COMPLETION REPORT

EFFECTS OF HANDLING AND CROWDING ON THE STRESS RESPONSE AND VIABILITY OF CHINOOK SALMON PARR AND SMOLTS

by

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to

Department of Energy
Bonneville Power Administration
Division of Fish and Wildlife (PJ)
P.O. Box 3621, Portland, OR 97208

Project 82-5
Contract DE-AC79-83BP11196

Gerald R. Bouck, Project Officer

ACKNOWLEDGMENTS

We thank the persons and organizations that assisted us in this study. The staff of Dworshak National Fish Hatchery made wet laboratory space available to us for several months in 1982, and provided other assistance at that time. Experimental fish were provided by Dworshak, Kooskia and Hager-man National Fish Hatcheries and by the Rapid River (Idaho) State Hatchery. Dr. Wally Zaugg measured (Na + K)-ATPase concentrations in gill samples. Dr. Carl Schreck provided a procedure for radioimmunoassay of plasma cortisol samples. The Seattle National Fisheries Research Center provided space at Marrowstone Field Station and lent us a flame photometer for plasma sodium ion analysis. Laboratory space for radioimmunoassay of plasma cortisol was provided by the Department of Animal Science at the University of Idaho. Dr. Paul Eschmeyer of the U.S. Fish and Wildlife Service's Editorial Office suggested numerous improvements in the manuscript.

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SUMMARY

Transportation of migrating chinook salmon smolts from Snake River dams to the Columbia River estuary has not reversed a downward trend in Idaho stocks of this species that first became apparent in the late 1960s. Poor survival of transported smolts may be a consequence of physiological responses to stressful events during collection and transportation. This study was undertaken to evaluate the intensity of stress responses in transported smolts, to determine if stress responses decrease the viability of transported smolts, and to investigate ways of avoiding or mitigating stressful events during transportation.

Tests were carried out at Dworshak National Fish Hatchery (Dworshak NFH) to establish baseline concentrations for plasma cortisol, glucose and Na+ in spring chinook salmon under stressed and unstressed conditions. The fish were crowded at densities of 32, 64 or 128 g/liter (2, 4 or 8 pounds/cubic foot) for 2 to 96 hours. The stage of smoltification was estimated by migration tests and measurement of gill (Na + K)-ATPase activity.

Cortisol was undetectable (<3 ng/ml) in plasma of uncrowded spring chinook salmon sampled in March, but ranged from 29 to 98 ng/ml in plasma of the same and three additional groups of fish sampled in April, May, or June. These and other cited data indicate that plasma cortisol concentrations rose during smoltification.

Plasma cortisol concentrations were higher in smolts than in parr at each of the three crowding densities tested. Glucose concentrations were higher and Na+ concentrations were lower (i.e. stress response was stronger) in smolts

than in parr at the two higher crowding densities and at longer (48- and 96-hour) test durations.

Concentrations of stress indices returned toward control concentrations more rapidly in chinook salmon (parr and smolts) transferred to sea water (20°/oo) after crowding than in those transferred to fresh water. Recovery in sea water was usually complete in 24 hours: recovery in fresh water was often incomplete after 72 hours.

In 1982, spring chinook salmon were sampled at seven points in the collection process at Lower Granite Dam and after transportation to Bonneville Dam by truck or barge. On three of the four sampling dates, plasma cortisol concentrations in fish from gatewells were similar (40-60 ng/ml) to baseline cortisol concentrations in hatchery-reared chinook salmon: cortisol concentrations were higher (135, 154 ng/ml) in fish from gatewells on the fourth sampling date (April 30). A die1 cycle in both cortisol and glucose levels in chinook salmon exiting the bypass pipe indicated that the stress response became progressively more pronounced in fish that could not find their way out of the gatewells or bypass system. Concentrations were highest in fish passing through the bypass at the peak of the evening migration, and lowest in fish exiting the gatewells at midday (and presumably delayed since the previous evening).

Passage from gatewells to raceways resulted in a large change in stress indices relative to most other steps in the collection and transportation process. Overall mean cortisol concentrations were 112 ng/ml in fish exiting the bypass and 160 ng/ml in fish entering raceways. Only loading on trucks and barges elicited an equally large increase in plasma cortisol response. Fish swam vigorously in the bypass, as indicated by an elevated plasma lactate

concentration (67 mg/lOO ml, compared with 24 mg/lOO ml after overnight recovery in a raceway).

Plasma cortisol concentrations dropped rapidly during the first 6-8 hours of raceway residence, then rose again after smolts were loaded onto trucks or barges (overall mean cortisol concentration after loading, 175 ng/ml). Concentrations declined during barge transportation on three of the four dates sampled, but rose on the last sampling date (April 30). Plasma cortisol remained elevated in the one group of trucked fish sampled at Bonneville. Plasma glucose concentrations increased (to highs of 135-145 mg/lOO ml) and Na+ concentrations decreased (to lows of 122-132 meg/liter) during collection and transportation.

Plasma cortisol concentrations returned to the baseline range (<60 ng/ml) within 24-48 hours in chinook salmon transported to Bonneville by truck and held in fresh water. Plasma glucose and Na+ concentrations recovered more slowly, but fish could tolerate full strength sea water within 24 hours or less.

Vulnerability to predation increased in all stocks of spring chinook salmon tested after the fish were crowded at high densities (193 and 385 g/liter) and long durations (96 and 240 hours). Predation rates and plasma cortisol concentrations were significantly correlated. Chinook salmon with plasma cortisol concentrations of 75-150 ng/ml or higher were captured by predators at a higher rate than were control fish. Plasma cortisol concentrations in transported chinook salmon frequently exceeded 150 ng/ml at the time of release. The physiological response to stressors associated with transportation probably would impair the ability of transported fish to escape predators immediately after release, but losses would depend on numbers of predators near release sites.

The effects of truck transportation and of exposure to an additional stressor (30 seconds out of water in a net) on later survival in sea water were evaluated. High survival (90%-98%) of chinook salmon smolts during l0-19 weeks of seawater rearing was correlated with high survival during previous freshwater rearing in the hatchery. The 30 seconds of exposure to air at the time of truck unloading did not decrease survival of transported fish. Seawater survival was not closely correlated with the estimated incidence of bacterial kidney disease (by fluorescent antibody technique) in fish before transfer to sea water, but almost all of the fish that died in sea water had typical symptoms of the disease.

The planned release of marked stressed and unstressed chinook smolts from Eagle Creek National Fish Hatchery (Eagle Creek NFH) was not achieved in 1983. A release of marked "stressed" and "unstressed" fish was made in spring 1984. Comparison of adult return rates from the two groups should indicate whether exposure of smolts to a strong stressor before release to stress adversely affects survival.

After chinook salmon smolts *were* transported and handled, plasma cortisol declined more rapidly in smolts held in 5°/oo or 10°/oo sea water than in those held in fresh water or 20°/oo sea water. Recovery of depressed plasma Na^+ concentrations after transportation was slowest in fish held in fresh water and fastest in fish held in 20°/oo sea water. Use of 5°/oo sea water (or salt water) during transport of fish by truck should minimize mortality due to stress-caused ionoregulatory disturbances. Release of barged chinook smolts into brackish water should speed recovery of stress hormone and blood electrolyte concentrations.

1. INTRODUCTION

Runs of spring chinook salmon have been declining in Idaho since the late 1960s. By the 1970s, the adult return rate of smolts released from the Idaho Fish and Game Department's Rapid River Hatchery had declined from about 0.8% to 0.1% or below (Fig. 1.1). This decline in survival rates coincided with an increase in the number of operating turbine units on the Snake River from 3 in 1968 to 24 in 1979 (Fig. 1.1). In an attempt to reduce mortalities caused by turbine passage and by predation, disease, and delay in the eight reservoirs on the Snake and Columbia Rivers, transportation of smolts from the Snake River to the lower Columbia River was begun in the mid 1970s (Fig. 1.1). Traveling screens divert migrating chinook salmon and steelhead trout smolts from turbine intakes at Lower Granite and Little Goose dams, the two uppermost dams on the lower Snake River, into a bypass system. The fish are held in raceways, and then loaded into either fish transport tankers or barges for transport to the estuary. Results of the transportation program have been encouraging for steelhead trout, but disappointing for chinook salmon. Although 50% or more of the chinook salmon migrating down the Snake River have been transported each year since 1977 (Fig. l.l), return rates have continued to be very poor. The existence of Idaho chinook salmon stocks will soon be in jeopardy unless return rates are substantially improved: consequently, the failure of the transportation program is a matter of serious concern.

We believe that the poor post-release survival of transported smolts may be related to the physiological effects of events they experience during collection and transportation. The stress response in fishes is characterized by disturbances in endocrine, metabolic and osmoregulatory function that either can be directly lethal

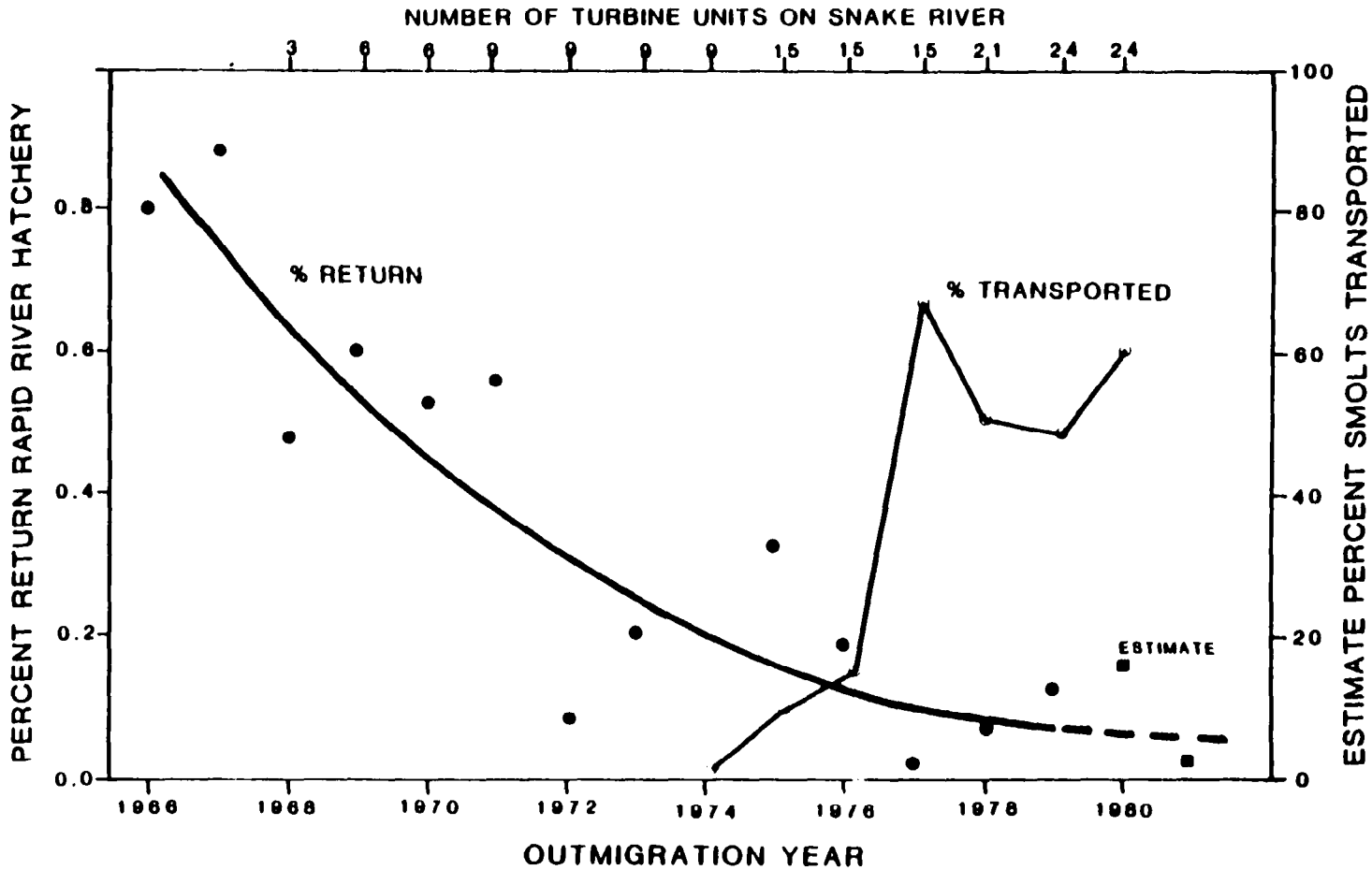

Figure 1.1. Survival rate of chinook salmon smolts released from Rapid River hatchery, percentage of smolts passing Lower Granite and Little Goose dams that were collected and transported, and the number of turbines installed in lower Snake River dams.

(Barton et al. 1980; Strange et al. 1977) or can reduce ability to respond to environmental challenges. The common factor mediating non-specific physiological responses to stressors is thought to be psychological distress, which is integrated with neuroendocrine activity via the hypothalmo-pituitary axis (Mason 1975). Experimental work with salmonid fishes has shown that handling and crowding can elicit a strong stress response (Barton et al. 1980; Strange et al. 1978; Wedemeyer 1976). Smolts entering the collection facility at Lower Granite or Little Goose Dams are exposed to a series of potentially traumatic events, including handling and crowding.

In 1982 the Bonneville Power Administration funded a two-year study to investigate the possible linkage between stress and decreased viability of transported chinook smolts. Research objectives for 1982 and 1983 were to:

1. Develop baseline information on the stress response in Parr, smolts, and post-smolts of chinook salmon.

2. Determine relative intensity of the stress response (as indicated by changes in physiological indices) associated with each step in the collection and transportation procedures at Lower Granite Dam.

3. Determine if changes in plasma cortisol in chinook salmon are correlated with decreased ability to avoid attacks by predatory fishes.

4. Determine whether collection and transportation of chinook salmon activates sub-clinical bacterial kidney disease infections and later affects survival during extended saltwater rearing, and how survival is affected by overall fish health.

5. Test the hypothesis that exposure of chinook salmon smolts to stressful conditions before they enter salt water adversely affects adult return rates.

6. Determine if a stress response can be avoided or mitigated by releasing smolts in dilute salt water after transportation.

2. EFFECTS OF CROWDING ON PLASMA CORTISOL, GLUCOSE, AND NA+ CONCENTRATIONS IN CHINOOK SALMON PARR, SMOLTS, AND POST-SMOLTS

A primary objective of this project was to determine the physiological response of migrating chinook salmon smolts to collection and transportation procedures encountered at Lower Granite Dam. Changes in plasma cortisol, glucose and electrolytes are characteristic features of the physiological response of fish to many types of stressors (Mazeaud et al. 1977; Strange et al. 1977). Because adequate information was not available on expected concentration ranges of plasma cortisol, glucose, and electrolytes in juvenile chinook salmon before and after exposure to stressors a study was undertaken to obtain this information. Crowding was selected as a stressor because crowding densities are both easily defined and reproducible.

Strange et al. (1978) reported plasma cortisol concentrations in chinook salmon stressed by confinement and crowding, but did not investigate the interactive effects of stressor intensity (crowding density) and duration. The effect of smoltification on the stress response in salmonid fishes had not been specifically investigated prior to the present study, although Wedemeyer (1976) observed more extreme blood glucose changes in coho salmon smolts than in parr following netting and handling.

The objectives of the present study were to determine changes in plasma cortisol, glucose and Na+ in spring chinook salmon subjected to several crowding densities for various periods, and to determine recovery rates for these variables when fish were transferred to fresh water and salt water after completion of crowding trials. The effects of smoltification and of stressor intensity-duration

interactions on changes in monitored variables and on rates of recovery were of particular interest.

Methods and Materials

Selection and Maintenance of Test Groups

Eight groups of spring chinook salmon were used in baseline stress response studies at Dworshak NFH in 1982 (Table 2.1). These groups were selected to represent a range of rearing histories and degrees of severity of bacterial kidney disease (BKD). Dworshak spring chinook salmon (age 1, reared in unheated water) were tested three times (late March, mid April and late June) to determine the effect of the parr/smolt transformation within a cohort on the stress response. A group of age 0 fall chinook salmon were also tested.

Incidence of BKD and degree of smoltification were estimated for each group. Fecal or frozen whole-body samples were taken for later determination of BKD infection by the fluorescent antibody technique. Smolt status was estimated on the basis of three factors: branchial (Na + K)-ATPase activities (determined by W. Zaugg), physical appearance, and migratory activity. Migratory activity was tested in an 80-m concrete channel divided by dam boards into four pools. Groups of 100 branded fish were periodically introduced into the uppermost pool, and fish that migrated were recovered in a trap below the lowermost pool. Migration tendency was expressed as the percent of stocked fish that migrated per day during the period of the test.

"Indices of smoltification" are notoriously inaccurate when applied to spring chinook (Ewing and Birks 1982), because the developmental sequence of this species is so

Table 2.1. Characteristics of experimental groups of chinook salmon (spring run unless otherwise indicated) used in baseline stress-response testing at Dworshak National Fish Hatchery, spring 1982. ATP is gill (Na + K)-ATPase activity (micro-mol P/mg protein per hour), and MT is migration tendency (percent stocked fish migrating from test channel per day). REL-ATP and REL-MT are, respectively, the relative ATPase activity and the relative migration tendency, both expressed as a fraction of the highest values observed in any group. SI, the "smoltification index," is the sum of REL-ATP and REL-MT. Percentages of fish found positive for bacterial kidney disease (BKD) by the fluorescent antibody technique are indicated.

Group origin	Abbreviation[a]	Total length, range (mm)	Age	Dates tested	ATP (REL-ATP)	MT (REL-MT)	SI	BKD (%)	Stage
1. Mixed migrants, Lower Granite Dam	MM	100-190	1	Apr 24 - May 1	-----	-----	----	----	Smolt
2. Hagerman National Fish Hatchery[b]	HF	100-120	0	Jun 7 - 17	46.04 (1.0)	6.3 (1.0)	2.00	0.0	Smolt
3. Dworshak National Fish Hatchery[c]	DLS	160-190	1	Apr 7 - 20	27.16 (0.59)	4.7 (0.75)	1.34	58.8	Smolt
4. Dworshak National Fish Hatchery	DSS-3	140-170	1	Jun 20 - 30	17.03 (0.37)	3.5 (0.55)	0.92	70.8	Smolt
5. Dworshak National Fish Hatchery	DSS-2	120-140	1	Apr 17 - 29	7.83 (0.17)	1.3 (0.25)	0.42	70.8	Parr
6. Dworshak National Fish Hatchery	DSS-1	100-130	1	Mar 22 - Apr 3	5.52 (0.12)	0.0 (0.0)	0.12	70.8	Parr
7. Kooskia National Fish Hatchery	KS-1	110-140	1	Apr 12 - 20	9.21 (0.20)	----- (---)	----	22.4	Parr
8. Kooskia National Fish Hatchery	KS-0	100-130	0	Jun 20 - 30	23.94 (0.52)	0.9 (0.14)	0.66	----	Parr
9. Rapid River State Hatchery	RR	100-150	1	Apr 1 - 7	12.43 (0.27)	----- (---)	----	1.5	Parr

[a] Abbreviations are used to identify data for individual groups shown in figures (results section).

[b] "Fall-run" fish.

[c] These fish were reared in reused and reconditioned water supplemented with electrolytes (20 ppm Na^+, 8 ppm K^+, 30 ppm Cl^-).

variable relative to other anadromous salmonids (e.g., Atlantic salmon, coho salmon and steelhead trout). If a smolt is defined as the developmental stage at which migration to the sea results in the highest adult returns, then "smoltification indices" vary greatly between different runs of chinook salmon. Among anadromous salmonids whose transformation from parr to smolt is distinct and temporally compressed, the most reliable indicators of the occurrence of smoltification are probably: (1) the development of high levels of activity of the enzyme (Na + K)-ATPase (which promotes hyperosmotic adaptation), (2) the acquisition of tolerance to full-strength sea water, (3) the loss of territoriality, and (4) the adoption of a downstream migratory disposition (Hoar 1976, Folmar and Dickhoff 1980). Various populations of chinook salmon, however, may migrate downriver with or without elevated ATPase activities (Ewing et al. 1980). Moreover, salinity tolerance bears no relationship to smoltification in chinook salmon, as any fish of 80 mm or more total length tolerates full-strength sea water (Ewing and Birks 1982). Indeed, as stated by Ewing and Birks (1982), "the various components normally associated with the smolting process--physiological changes, migration, ocean entry and survival to adulthood--may be separated temporally in chinook salmon, and the concept of 'smolt,' as defined in other species, may not be applicable." Nevertheless, Ewing and Birks presented evidence of a correlation between the time of maximally elevated ATPase activity and the time of downstream migration and ocean entry that resulted in the highest adult returns in Rogue River spring chinook. The most reliable index of "smoltification" in chinook salmon seems to be the simultaneous development of high levels of ATPase activity and a strong disposition to migrate downstream.

The relative degree of smoltification of fish used in this study was assessed by comparing physical appearance,

branchial ATPase activity, and migratory tendency. Fall chinook salmon reared at Hagerman National Fish Hatchery (Hagerman NFH) had both the highest ATPase activity (46.04 micro-mol P per mg protein per hr) and the highest migration tendency (6.3% per day) observed in any group. To facilitate comparisons of smolt status between groups, the ATPase activity and migration tendency of each group was expressed as a fraction of the values observed in Hagerman fall chinook salmon. These relative ATPase and migration tendency values were then summed to give a composite "smoltification index" (SI; Table 2.1).

On the basis of the SI and the acquisition of a "smolt-like" appearance resulting from the loss of parr marks and the development of silvery coloration and slim body form, Hagerman fall chinook salmon and Dworshak large spring chinook salmon were considered to be smolts when sampled in June and parr when sampled in March or April. The remaining groups of spring chinook salmon from Kooskia National Fish Hatchery (Kooskia NFH) and Rapid River Hatchery were considered parr because either ATPase activity or migratory tendency, or both, were low.

All tests were carried out in the incubation room of Dworshak NFH under constant artificial illumination (incandescent light, intensity not measured). Filtered, heated water from the North Fork of the Clearwater River was supplied to the test and holding tanks at a mean temperature of 10.5 C (9.4-11.6 C). Fish were held in 1.2- or 1.8-m diameter circular tanks for a minimum of 4 days prior to testing. They were fed Oregon Moist Pellets (3% of body weight divided between two daily feedings) until 2 days before testing, and then were fasted through completion of the test.

Sampling Procedure

Blood samples were taken by dip-netting groups of 5 fish into a plastic bucket filled with 3 liters of unbuffered tricaine methanesulfonate (MS-222) at a concentration of 50 ppm. (Preliminary studies indicated that plasma glucase, cortisol and electrolytes were not appreciably affected by short periods of exposure to unbuffered MS-222 at 50 mm.) Fish were blotted dry and measured after they became anesthetized. Caudal peduncles were severed and blood was collected in heparinized Caraway tubes (370 microliters capacity, ammonium heparin coated), pooled (blood from 5 fish), and centrifuged to separate blood cells and plasma. The plasma was divided between two 250-microliter sample cups (one for glucose and electrolyte analysis, the other for cortisol analysis), and frozen at -20 C for storage. A sample consisted of three 5-fish replicates (n = 15), except for saltwater recovery samples, which consisted of four 5-fish replicates (n = 20). Total time in MS-222 (usually less than 5 minutes) and sampling order (replicate 1, 2, or 3) were recorded for each replicate.

Sample Analysis

The physiological variables monitored were plasma cortisol, glucose and Na^+. Plasma cortisol was determined with a commercial radioimmunoassay kit (Biorad "quantimune" cortisol)'. The within-run coefficient of variation (C.V.) was calculated at 8% and 13% in two runs (n = 10 each run); the between-run C.V. ranged from 15% (58 ng/ml control pool: n = 12) to 24% (255 ng/ml control pool; n = 12). Percent recovery of added human cortisol was 75% in one test and 100%

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1 Reference to a commercial product does not imply endorsement of that product by the U.S. Government.

in a second test. Comparison of cortisol values for nine samples analyzed at the University of Idaho with values for the same samples analyzed at Oregon State University indicated that values obtained at the University of Idaho were significantly higher (signed rank test, $P < 0.025$: $\bar{x}$ difference = +24%.

Plasma glucose was measured with a commercial enzymatic kit (Sigma Chemical 115A). In this procedure, glucose is converted to glucose-6-phosphate in the presence of hexokinase, with concurrent reduction of NADP to NADPH. The resulting increase in concentration of NADPH is determined spectrophotometrically at 520 nm. Within-run and between-run coefficients of variation (n = 8 and 6, respectively) were 10%. The reduction in glucose concentration resulting from repeated freezing (to -15 C) and rethawing over four freeze-thaw cycles was 13% (n = 4 control pools).

Plasma Na^+ was measured with either an inductively coupled plasma spectrophotometer (ICPS; Applied Research Laboratories model 143) or a flame photometer (Instrumentation Labs model 142). The within and between-run coefficient of variation for Na^+ when the flame photometer was used was 5%.

Test Protocols

Loading-Density-Duration Tests. Fish were stressed by netting them from their holding tanks into floating mesh cages in which the volume had been adjusted to give loading densities of 32, 64, or 128 g/liter (2, 4, or 8 pounds per cubic foot). Test fish were then left in crowding cages for various periods of time (2, 8, 24, 48, or 96 hours). Crowding durations extended up to 96 hours because the total duration over which fish are collected at Lower Granite Dam and transported downriver frequently equals or exceeds 96

hours. Loading densities were multiples of 64 g/liter because this density is the greatest density used during transport. Styrofoam lids were strapped to the tops of the crowding cages, which were than allowed to float free in the holding tanks (1.2- or 1.8-m diameter). Fish that remained free in the holding tank for the duration of the experiment were never loaded in excess of 0.8 g/liter, and were used as controls. Fresh water at an average temperature of 10.5 C was circulated through the holding tank throughout the experiment.

Fish from some hatcheries were not tested at all combinations of loading density and duration. The minimum protocol employed only the 64 g/liter loading and durations of 2, 8, 24, and 96 hours.

Recoverv in Sea Water. At the conclusion of selected loading density-duration tests at 64 g/liter, lots of 30 test fish were transferred to each of two plastic cans containing 70 liters (19 gallons) of natural or artificial ("Marine Environment") sea water at a salinity of 20°/oo. The cans were aerated and maintained at the same temperature as the holding and crowding cages. Fish were quickly transferred from the crowding cages to the cans by dip-netting them from the cage into a bucket of sea water and then pouring the bucket into the can, so that they were lifted from the water for only 1 or 2 seconds. At 24, 48, and 72 hours after transfer to the cans of salt water, four 5-fish replicate samples were alternately netted from each of the cans. Uncrowded fish transferred from the holding tank into 20°/oo sea water served as controls.

Recoverv in Fresh Water. At the conclusion of selected loading density-duration tests at 64 g/liter, groups of 15 fish were transferred into each of three 190-liter (50-gallon) tanks supplied with flowing fresh water (same source

as holding tanks). The method used to transfer fish from crowding cages to recovery tanks was identical to that described above for groups recovering in salt water. One of the lots of fish was sampled at 24 hours, one at 48 hours, and the third at 72 hours. Physiological variables were compared with those of control fish.

Response to MS-222. Because fish were often in the MS-222 bath for periods up to 5 minutes--rarely up to 15 minutes--we determined the effect of exposure to unbuffered MS-222 on plasma glucose, cortisol and Na^+. Large Dworshak NFH spring chinook salmon (DLS) were dip-netted from a holding tank into an aerated, 50 ppm solution of unbuffered MS-222, and individual fish were removed and sampled after intervals ranging up to 60 minutes.

Statistical Analysis

Even when the fish were undisturbed, concentrations of plasma cortisol, glucose and Na^+ differed substantially among the various groups of fish tested. These differences were probably attributable to differences in developmental stage and size, prior hatchery or transportation experience, chemical composition of rearing water and, of course, genetic differences (e.g., spring versus fall chinook salmon). Thus, stress-induced differences in monitored variables between groups would be confounded with differences attributable to stress-independent factors if absolute concentrations were compared between groups. For this reason, inter-group statistical comparisons were made only in terms of "relative concentrations"--concentration changes relative to group-specific control values. For purposes of inter-group statistical comparison, the mean control concentration of each parameter was subtracted from the absolute concentration observed in test groups. The relative concentration changes so obtained were considered

to be deviations from group-specific control values, and statistical tests between groups assessed comparative degrees of deviation.

Data were analyzed by analysis of variance (ANOVA). Two-way factorial, randomized complete block (RCB) or one-way ANOVAs were used as appropriate. Multiple comparisons between treatment means were made by the PDIFF procedure of the Statistical Analysis System's General Linear Models program (Ray, Council and Sal1 1982). The alpha level for the null hypothesis of equal treatment means was 0.05.

Emphasis was placed on elucidating the effects of smoltification on the stress response and on determining how the stress response varied with duration at different stressor (crowding) intensities. Data on the stress response of individual groups were not treated: instead, smolt groups were contrasted with parr groups (see Table 2.1 for description of groups). Although statistical treatments were always made on relative concentrations, most of the values in the following subsection are given in terms of absolute concentrations. The relative deviations from baseline concentrations are, for the most part, discernible in plots of absolute concentrations. When such relationships are not apparent, relative concentrations were used.

Results

Plasma Cortisol Concentrations

Concentrations in Control Fish. Plasma cortisol concentrations in uncrowded, undisturbed chinook salmon differed between groups tested (Table 2.2). In five of the seven groups, mean concentrations fell in the range 19-98 ng/ml. In one group, the small Dworshak NFH spring chinook

Table 2.2. Mean plasma cortisol concentrations (absolute) in eight groups of chinook salmon sampled at DNFH in 1982. Fish were sampled prior to crowding; values obtained are the best estimate of "control" values.

Experimental group[a]	Concentrations (ng/ml)	
	Mean	S.E.
1. Migrants from L. Granite Dam, April	232.0	33.1
2. HNFH "falls", June	43.5	11.9
3. DNFH "large", April	97.9	19.3
4. DNFH "small", June	29.4	6.7
5. DNFH "small", April	36.2	6.7
6. DNFH "small", March	<1.0	<1.0
7. KNFH, June	41.7	9.1
8. KNFH, April	19.0	11.8

a Numbers correspond to numbers in Table 2.1, where characteristics of groups are described.

parr sampled in March (DSS-l), cortisol was undetectable (<1.0 ng/ml). In another group, the migrating smolts transported from Lower Granite Dam (MM), mean cortisol concentrations were much higher, declining in uncrowded fish from 340 ng/ml on the first day of testing to 185 ng/ml on the last day (overall mean = 232 ng/ml). Plasma cortisol concentrations in migrating spring chinook salmon smolts sampled from the gatewells at Lower Granite Dam in 1982 (discussed in section 3) were typically in the range 40-60 ng/ml (discussed in a later section), suggesting that fish transported to Dworshak NFH for testing never completely recovered from the cumulative stresses of capture and transport. If so, our estimates of unstressed control cortisol concentrations are not valid, and comparisons between the mixed migrant group and other groups must be qualified. If control cortisol concentrations were biased upward, a positive deviation from the putative control concentration in a given test group would be an underestimate of the "true" deviation.

In general, increases in plasma cortisol in response to crowding were greater in smolts than in parr (Figs. 2.1, 2.2, 2.3). Relative cortisol concentrations were significantly greater for smolts than for parr across all test durations at each of the three loading densities tested (RCB ANOVA).

Effect of Crowding Duration. Plasma cortisol was significantly elevated above control concentrations in all smolt groups and, to a lesser extent, in parr groups 2 hours after transfer to the crowding cages. No significant further increase occurred after 8, 24, and 48 hours at any loading, but at 96 hours a significant increase occurred in the groups held at 32 and 128 g/liter (RCB ANOVA across all groups, Table 2.3).

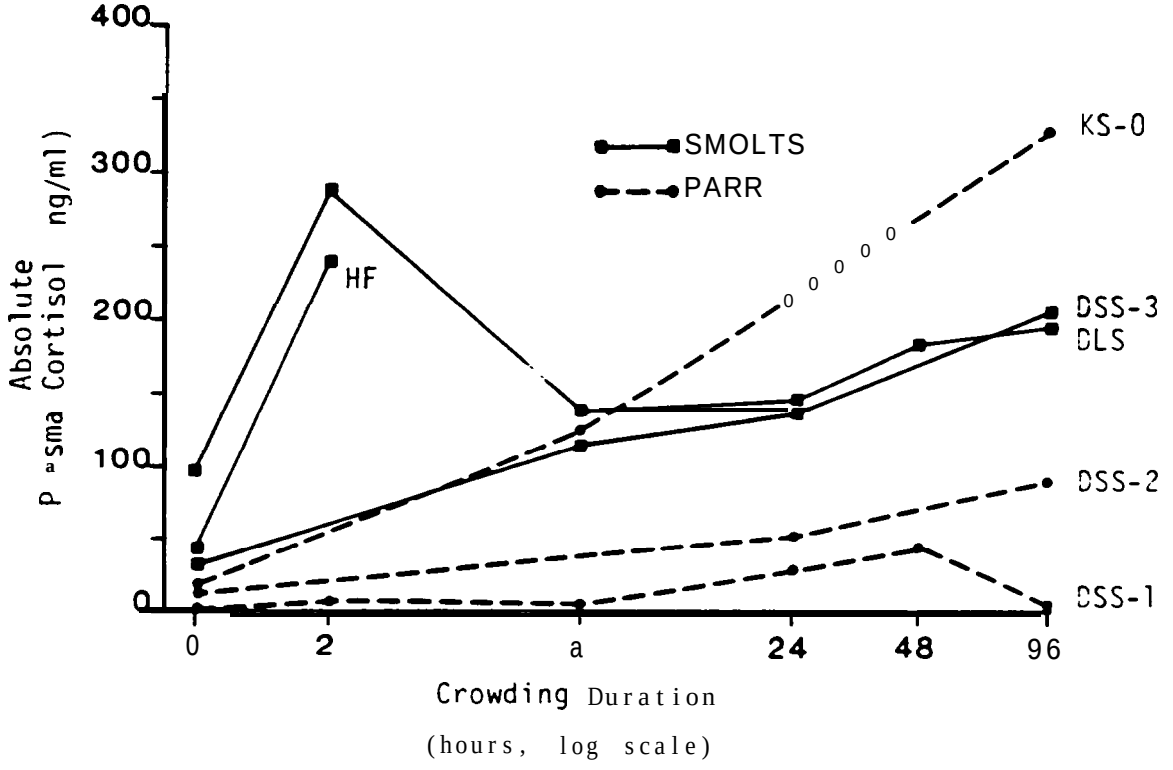


Figure 2.1. Plasma cortisol concentrations in six groups of spring chinook salmon crowded (32 g/liter) for 2 to 96 hours. Each point represents the mean of one to three pooled, five-fish replicate samples. Values at 0 hours are mean cortisol concentrations in uncrowded fish (controls). See Table 2.1 for description of groups of fish.

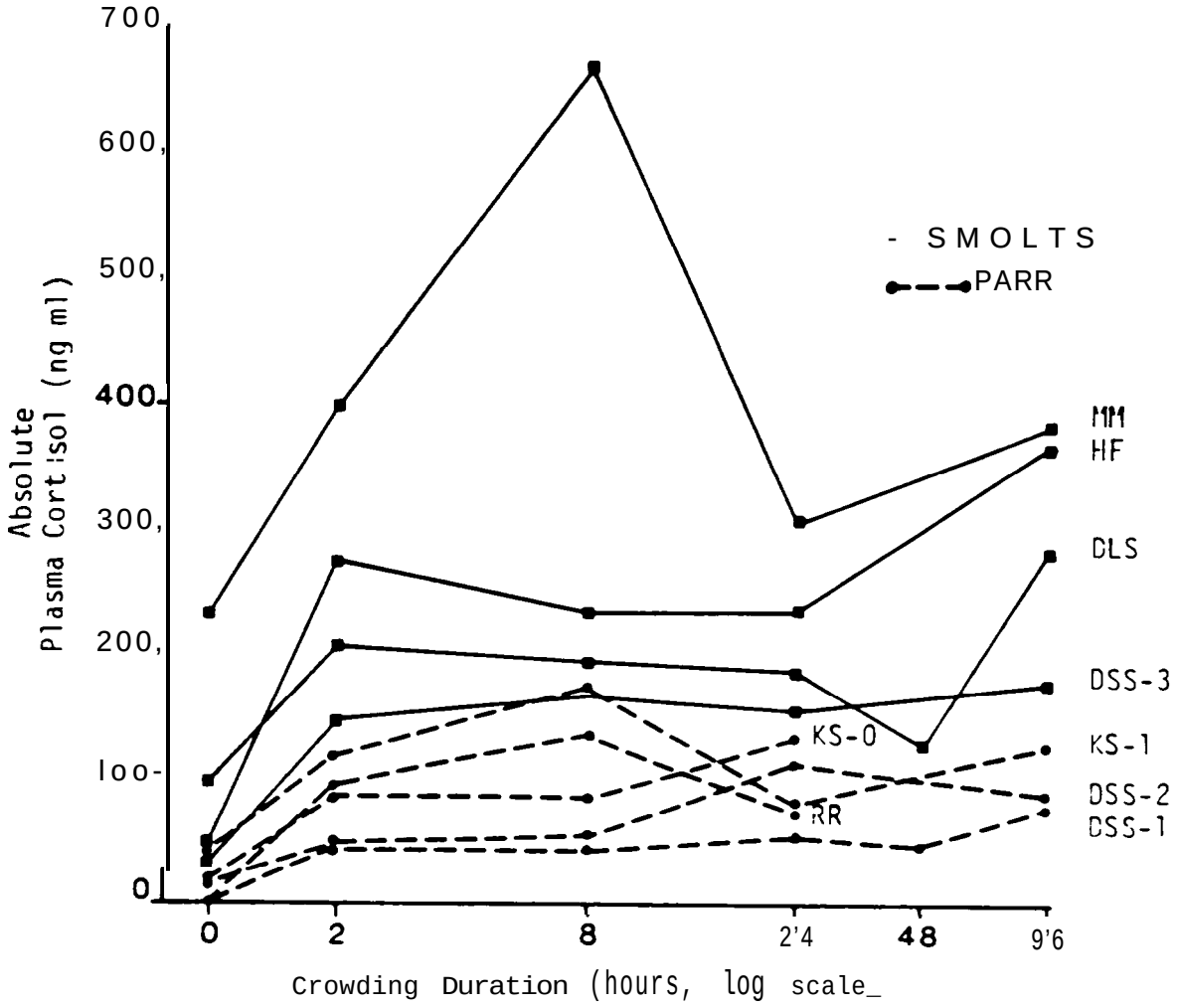


Figure 2.2 Plasma cortisol concentrations in nine groups of chinook salmon crowded (64 g//liter) for 2 to 96 hours. Each point represents the mean of one to three pooled, five-fish replicate samples. Values at 0 hours are mean cortisol concentrations in uncrowded fish (controls). See Table 2.1 for description of groups of fish.

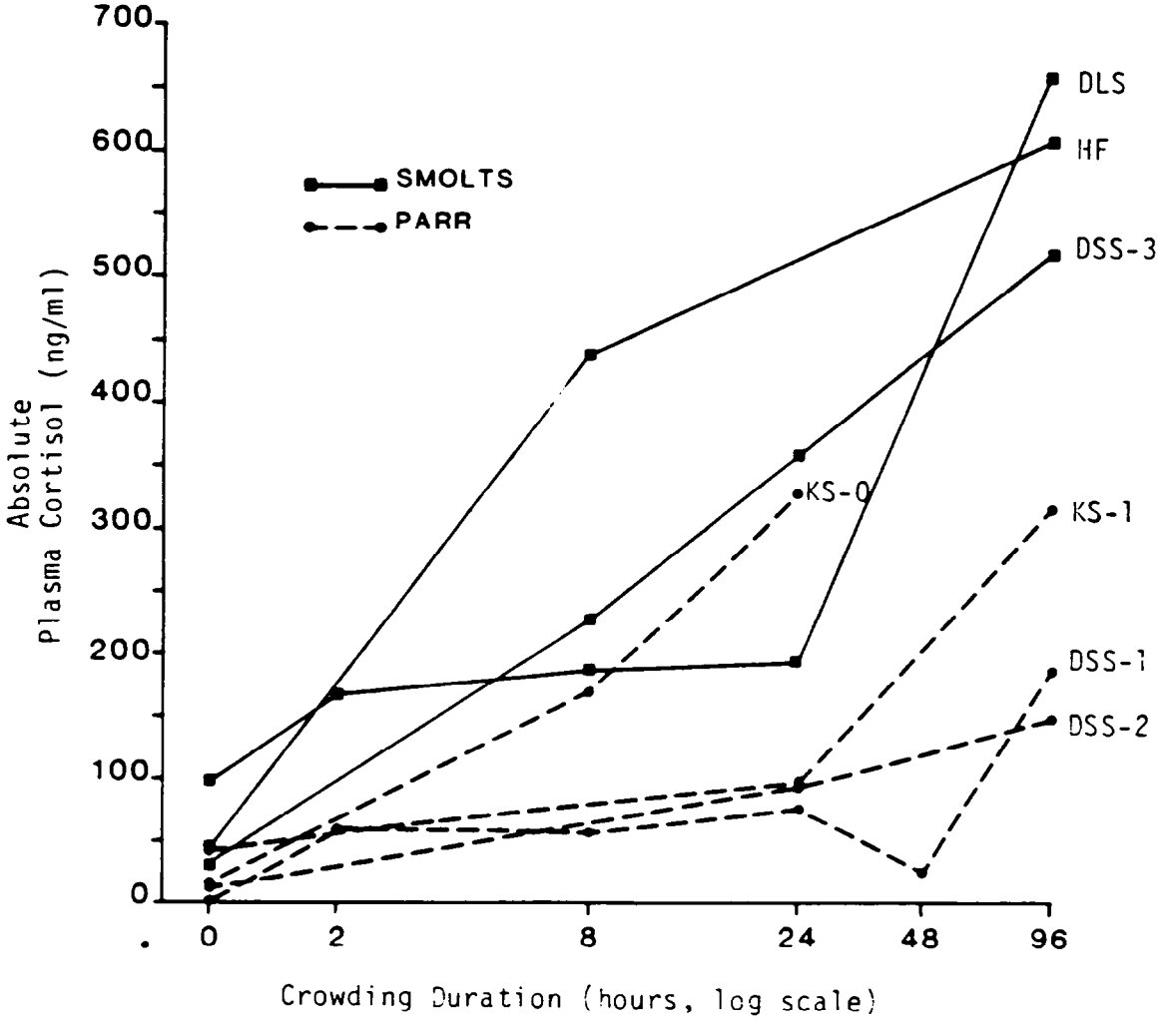


Figure 2.3. Plasma cortisol concentrations in seven groups of chinook salmon crowded (128 g/liter) for 2 to 96 hours. Each point represents the mean of one to three pooled, five-fish replicate samples. Values at 0 hours are mean cortisol concentrations in uncrowded fish (controls). See Table 2.1 for description of groups of fish.

Table 2.3. Comparison of mean (relative) plasma cortisol concentrations by randomized complete block ANOVA in chinook salmon (smolts and parr combined) crowded at three loading densities for various periods of time. Means that do not differ significantly are connected by a solid line. 0 hours = concentration in control fish.

Loading density (g/liter)	Crowding durations (hours)					
	0	2	8	24	48	96
32	—	—	—	—	—	—
64	—	—	—	—	—	—
128	—	—	—	—	—	—

Effect of Loading Density. An effect of increasing loading density on plasma cortisol concentrations was evident in smolt but not in parr. A one-way ANOVA of all relative cortisol concentrations in smolts, with load as treatment, indicated a significant difference between concentrations in fish held at 32 and 128 g/liter (concentrations in fish held at 64 g/liter were not significantly different from those held at either 32 or 128 g/liter). A similar comparison of relative cortisol concentrations in crowded parr indicated no significant differences at 32, 64, or 128 g/liter.

Plasma Glucose Concentrations

Baseline Concentrations in Control Fish. In six of the seven groups of chinook salmon tested, plasma glucose concentrations in uncrowded, undisturbed fish were in the range 58-91 mg/lOO ml (Table 2.4). A higher mean concentration (124 mg/lOO ml) in migrating smolts transported from Lower Granite Dam to Dworshak NFH was indicative of the previously mentioned prolonged stress response to capture, handling and confinement.

Smolts versus Parr. Plasma glucose did not increase to a significantly greater extent in smolts than in parr in response to crowding at 32 or 64 g/liter (Figs. 2.4, 2.5, 2.6; RCB ANOVA of relative concentrations across all durations). At 128 g/liter, relative glucose concentrations were significantly higher in smolts than in Parr; this difference was due to a greater increase in smolts than in parr after 48 and 95 hours duration (Fig. 2.6).

Effects of Crowding Duration. Plasma glucose was elevated significantly (except at 32 g/liter) above control concentrations (all groups) 2 hours after the fish were transferred to crowding cages, and increased progressively

Table 2.4. Mean plasma glucose concentration (absolute) in eight groups of chinook salmon sampled at DNFH in 1982. Fish were sampled prior to crowding, and values obtained represent the best estimate of "control" values.

Experimental group[a]	Concentrations (mg/lOOml) Mean	S.E.
1. Migrants from L. Granite Dam, April	124.0	13.1
2. HNFH "falls", June	74.2	6.8
3. DNFH "large", April	87.7	3.5
4. DNFH "small", June	91.2	5.1
5. DNFH "small", April	87.2	7.5
6. DNFH "small", March	77.9	3.3
7. KNFH, June	63.7	3.5
8. KNFH, April	58.2	12.0

a Numbers correspond to numbers in Table 2.1, where characteristics of groups are described.

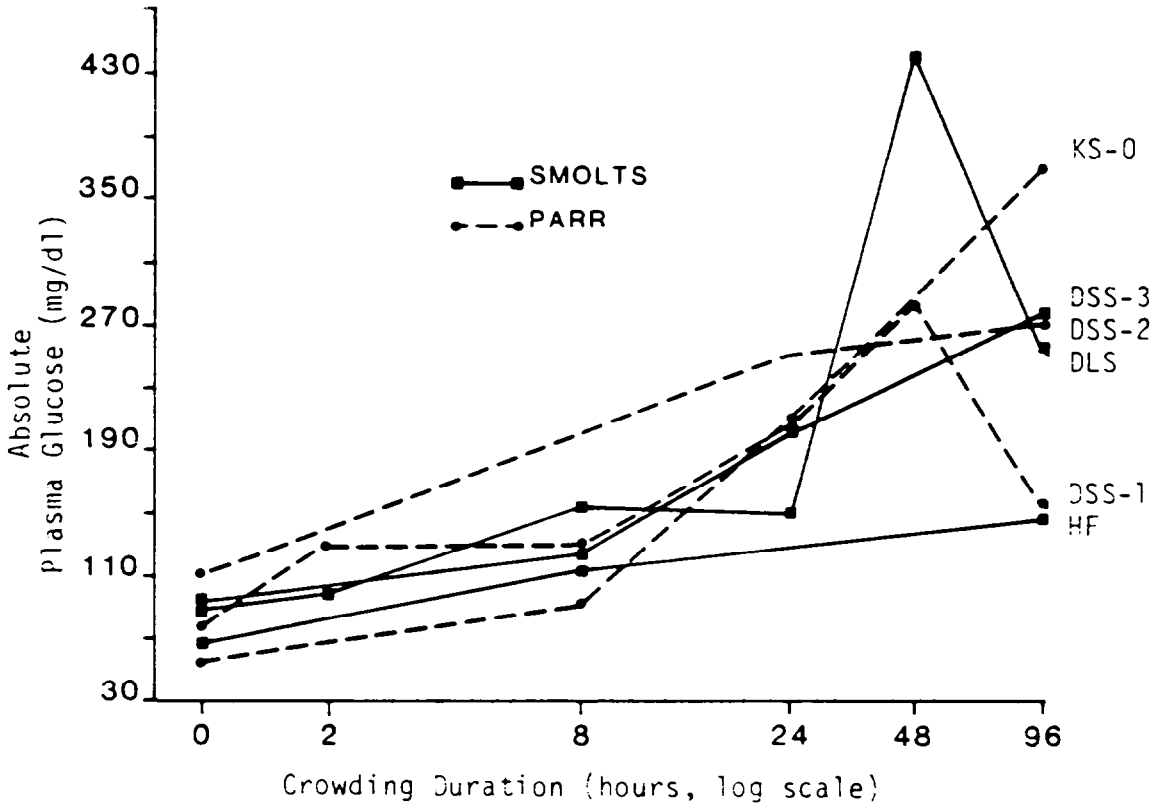


Figure 2.4. Plasma glucose concentrations in six groups of chinook salmon crowded (32 g/liter) for 2 to 96 hours. Each point represents the mean of one to three pooled, five-fish replicate samples. Values at 0 hours are mean glucose concentrations in uncrowded fish (controls). See Table 2.1 for description of groups of fish.

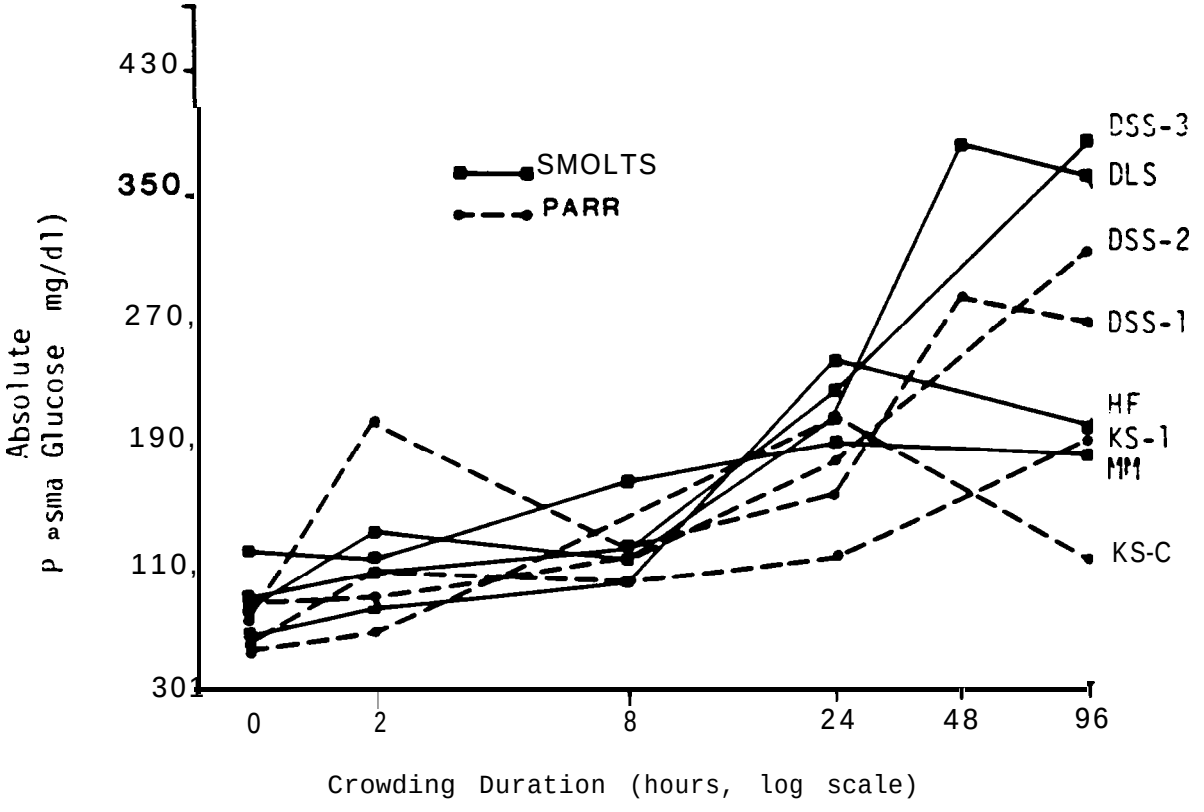


Figure 2.5. Plasma glucose concentrations in eight groups of chinook salmon crowded (64 g/liter) for 2 to 96 hours. Each point represents the mean of one to three pooled, five-fish replicate samples. Values at 0 hours are mean glucose concentrations in uncrowded fish (controls). See Table 2.1 for description of groups of fish.

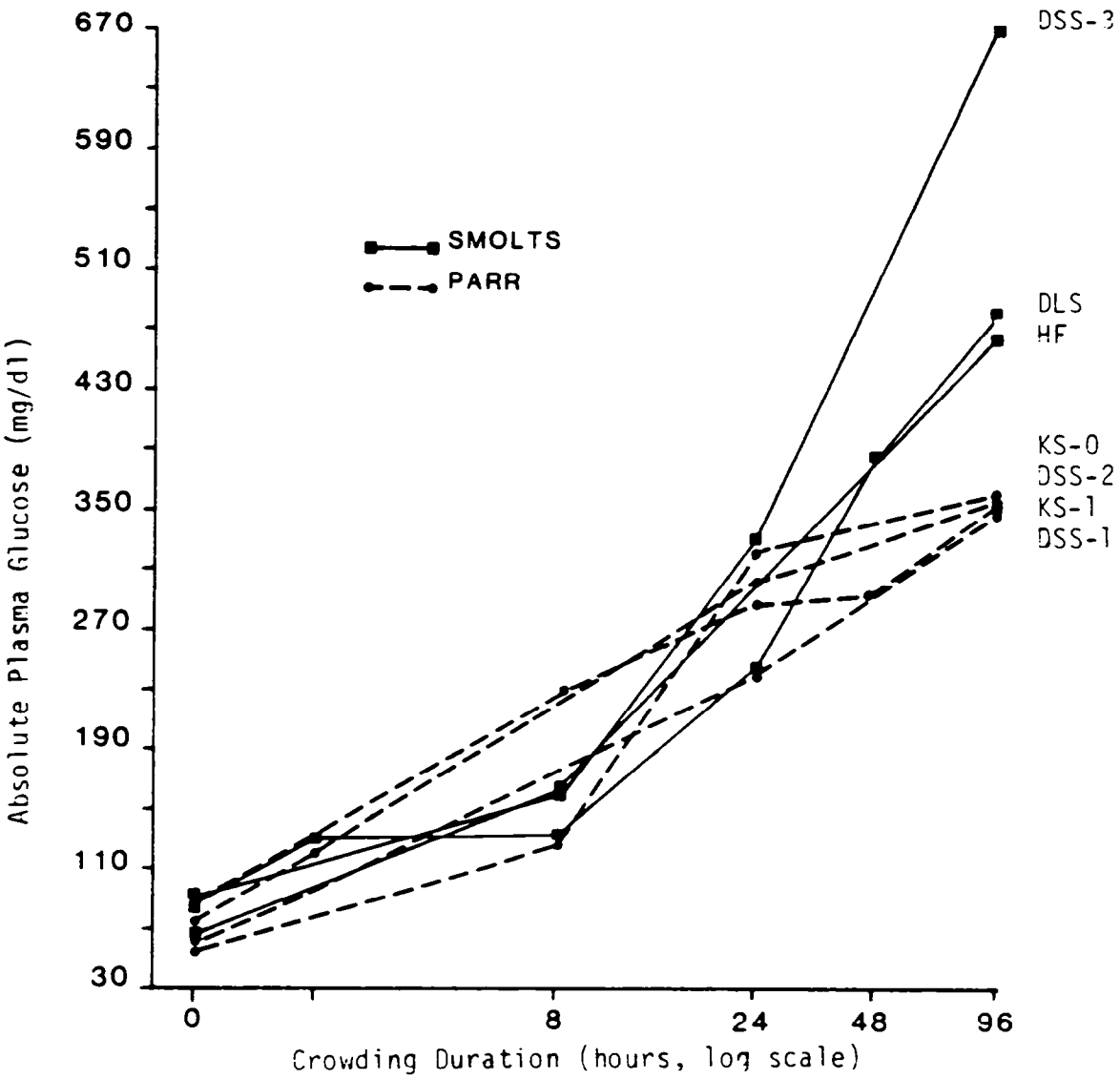


Figure 2.6. Plasma glucose concentrations in seven groups of chinook salmon crowded (128 g/liter) for 2 to 96 hours. Each point represents the mean of one to three pooled, five-fish replicate samples. Values at 0 hours are mean glucose concentrations in uncrowded fish (controls). See Table 2.1 for description of groups of fish.

with increasing duration (Figs. 2.4, 2.5, 2.6; Table 2.5) for 48 hours or longer. At the two lower loading densities, glucose concentrations in some groups declined between 48 and 96 hours. At the highest loading density (128 g/liter), plasma glucose continued to increase in all groups for the duration of the test, reaching 440-660 mg/lOO ml in the three smolt groups tested (Fig. 2.6).

Effect of Loading Density. Some effect of increased loading density on plasma glucose was evident in smolts, but the effect was equivocal in parr. A one-way ANOVA of all relative glucose concentrations in smolts with load as treatment indicated that concentrations did not differ significantly at 32 and 64 g/liter, but were significantly lower at 128 g/liter. A similar analysis for parr indicated that concentrations were not significantly greater in fish at 64 and 128 g/liter than in those at 32 g/liter, but were significantly greater in fish at 128 g/liter than in those at 64 g/liter (i.e., least square mean glucose concentration was greater at loading of 32 g/liter than at 64 g/liter).

Plasma Sodium Concentrations

Concentrations in Control Fish. Mean plasma Na^+ concentrations in undisturbed, uncrowded fish ranged from 142 to 180 meg/liter for the various groups tested (Table 2.6). The highest mean concentrations were in the two groups of fish with the highest indices of smoltification (Dworshak NFH large chinook and Hagerman NFH fall chinook: 180 and 165 meg/liter, respectively), disregarding the migrating smolts brought to the laboratory from Lower Granite Dam (142 meg/liter). As previously pointed out, we did not believe that the fish from Lower Granite Dam were fully recovered from capture and handling at the time of testing.

Tables 2.5. Comparison of mean plasma glucose concentrations by randomized complete block ANOVA in spring chinook salmon (all groups) crowded at three loading densities for various periods of time. Means that do not differ statistically from adjacent means are connected by a solid line. 0 hours = concentration in control fish. The downward arrow indicates a decrease from the preceding mean concentration.

Loading density (g/liter)	Crowding durations (hours)					
	0	2	8	24	48	96
32						↓
64						
128						

Table 2.6. Mean plasma Na^+ concentrations (absolute) in eight groups of chinook salmon sampled at DNFH in 1982. Fish were sampled before crowding, and values shown are the best estimate of "control" values.

Experimental group[a]	Concentrations (meg/liter)	
	Mean	S.E.
1. Migrants from L. Granite Dam, April	142	12.1
2. HNFH "falls", June	165	0.7
3. DNFH "large", April	180	5.8
4. DNFH "small", June	153	8.6
5. DNFH "small", April	156	5.5
6. DNFH "small", March	---	---
7. KNFH, June	15s	5.6
8. KNFH, April	155	4.7

a Numbers correspond to numbers in Table 2.1, where characteristics of groups are described.

Smolts versus Parr. Data on changes in plasma Na+ concentrations in crowded fish are given as relative concentrations (Figs. 2.7, 2.8, and 2.9) because the relative impact of crowding across groups was not apparent from plots of absolute concentrations. Plasma sodium concentrations initially rose in some parr, but then declined significantly after 24 hours of crowding at all loading densities. Little additional change occurred at 48 and 96 hours.

Effects of Crowding Duration. Relative plasma sodium ion concentrations did not decrease significantly from control concentrations 2 hours after transfer to crowding cages (Figs. 2.7, 2.8, 2.9: Table 2.7), but declined significantly by 24 hours and changed little thereafter.

Effect of Loading Density. Loading density had no significant effect on relative Na+ concentrations in either smolts or parr (one-way ANOVAs with all smolt groups and all parr groups, with load as treatment).

Recovery of Physiological Characteristics

Plasma Cortisol Concentrations. Figures 2.10 and 2.11 show changes in relative cortisol concentrations in smolts and parr recovering in fresh water and in salt water following crowding at 64 g/liter for 96 hours. (Again, plots of relative concentrations were used because the tendency of individual groups to restore concentrations of monitored variables to their respective control values is best displayed by such plots.) Data on recovery of parr and smolts recovering from crowding at 64 g/liter for 2, 8, and 24 hours followed similar trends and are not shown (recovery tests involved only fish crowded at 64 g/liter).

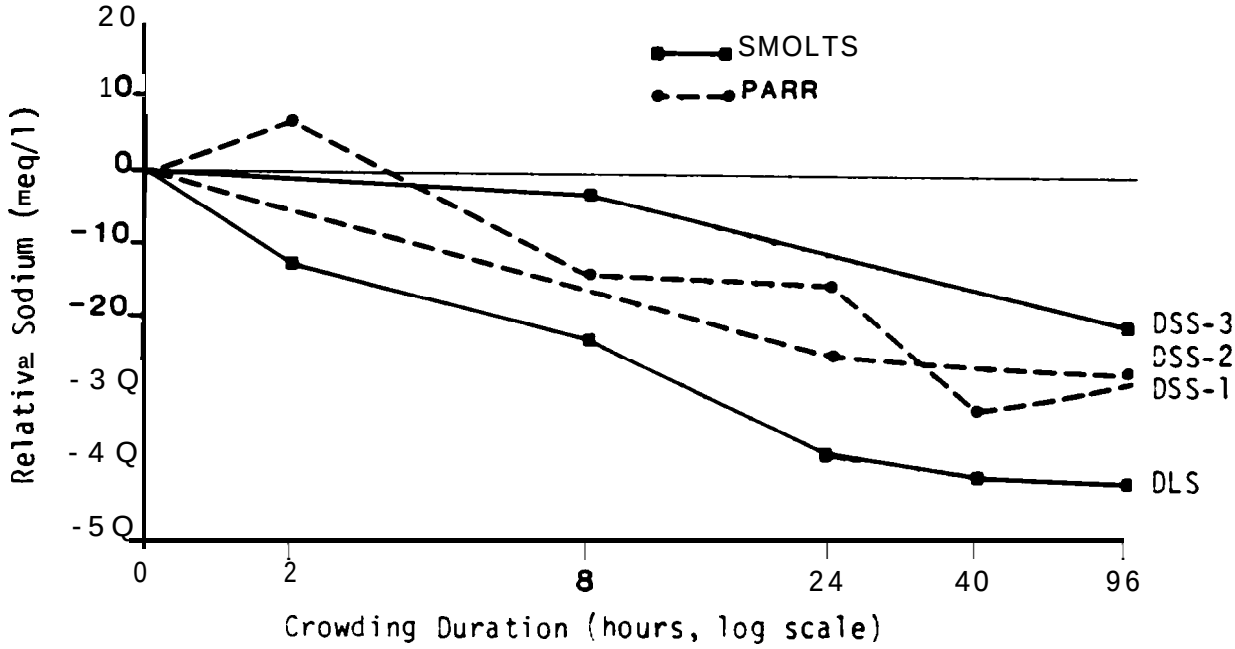


Figure 2.7. Relative plasma Na^+ concentrations (absolute minus control concentrations) in four groups of chinook salmon crowded (32 g/liter) for 2 to 96 hours. A concentration of zero (horizontal line) represents the control; negative concentrations indicate the degree to which test were lower than control concentrations. Points represent the mean of one to three pooled, five-fish replicate samples. See Table 2.1 for description of groups of fish.

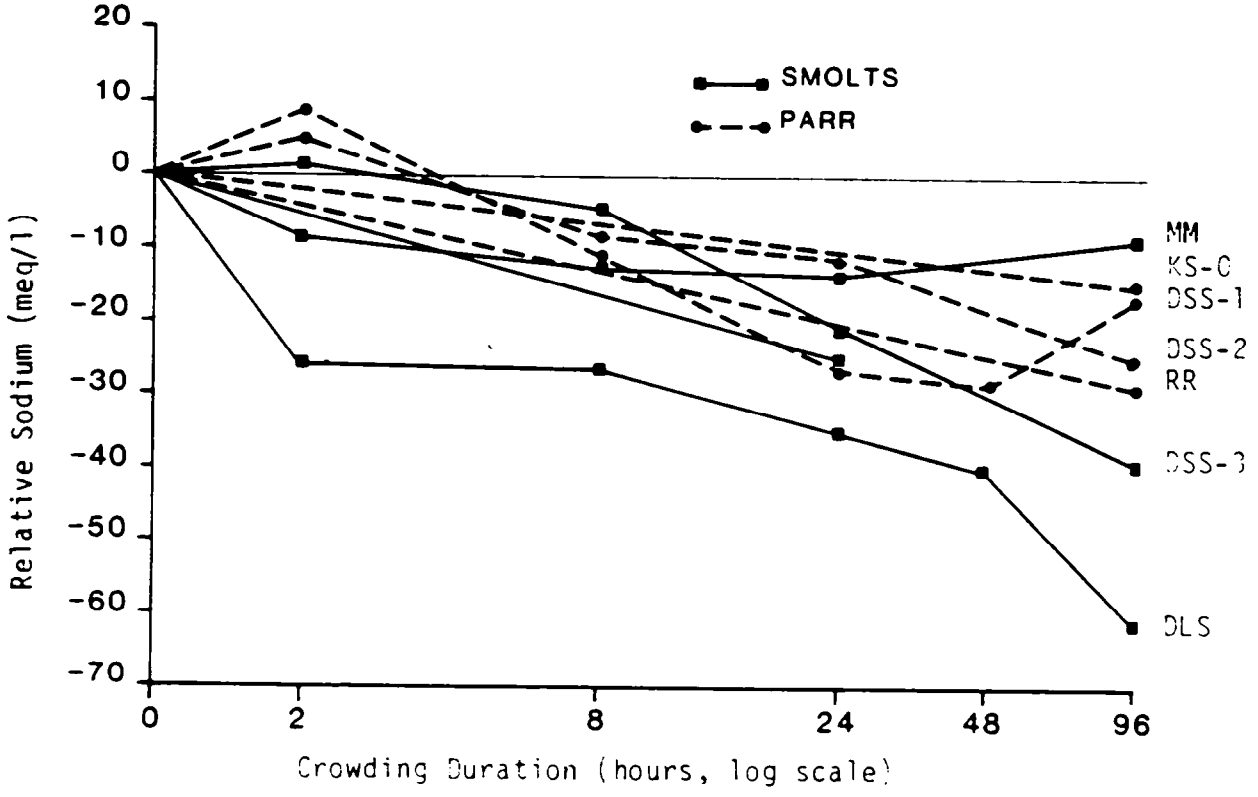


Figure 2.8. Relative plasma Na^+ concentrations (absolute minus control concentrations) in seven groups of chinook salmon crowded (64 g/liter) for 2 to 96 hours. A concentration of zero represents the control; negative concentrations indicate the degree to which test were lower than control concentrations. Points represent the mean of one to three pooled, five-fish replicate samples. See Table 2.1 for description of groups of fish.

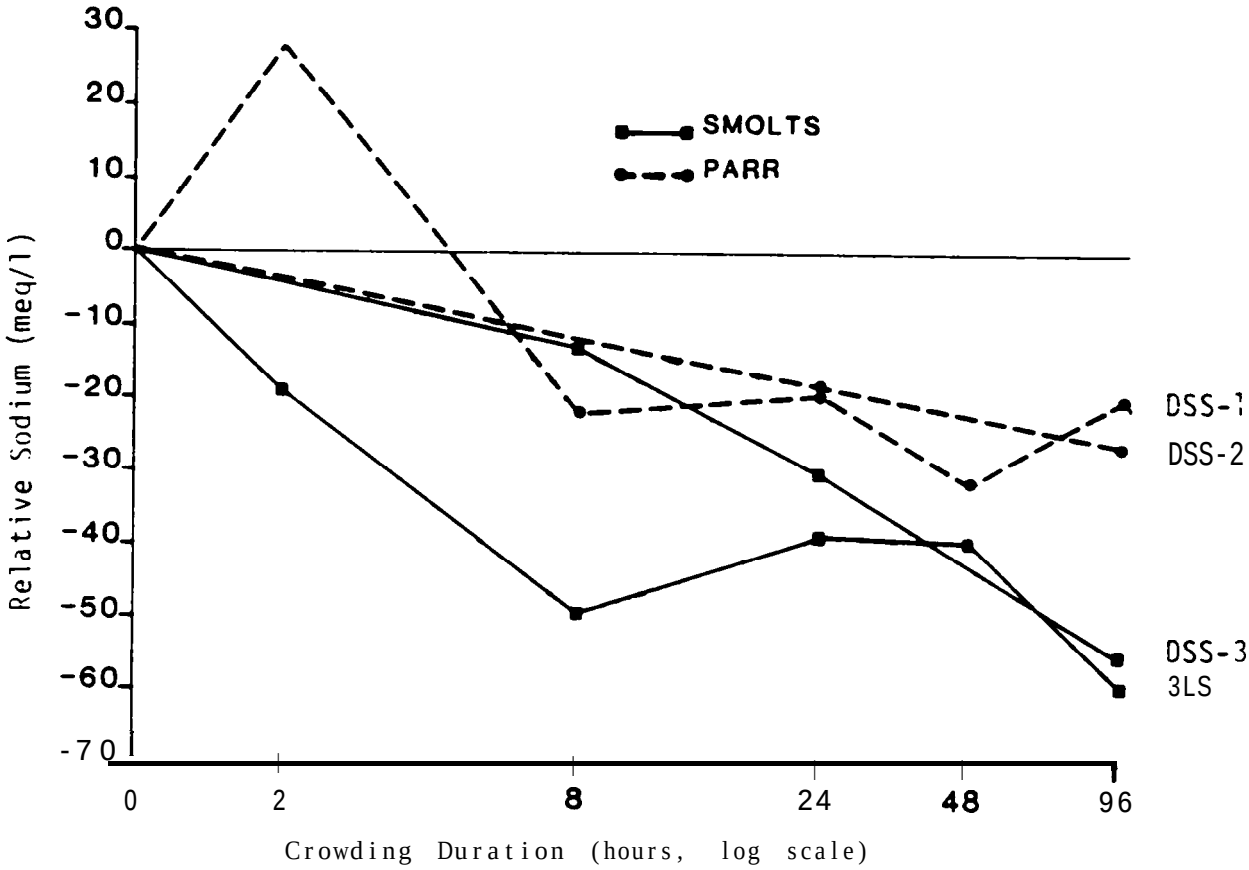


Figure 2.9. Relative plasma Na^+ concentrations (absolute minus control concentrations) in four groups of chinook salmon crowded (128 g/liter) for 2 to 96 hours. A concentration of zero represents the control; negative concentrations indicate the degree to which test were lower than control concentrations. Points represent the mean of one to three pooled, five-fish replicate samples. See Table 2.1 for description of groups of fish.

Table 2.7. Comparison of mean plasma Na+ concentrations by randomized complete block ANOVA in spring chinook salmon (all groups) crowded at three loading densities for different periods of time. Means that do not differ significantly are connected by a solid line. 0 hours = concentration in control fish.

Loading density (g/liter)	Crowding duration (hours)					
	0	2	8	24	48	96
32						
64						
128						

Plasma cortisol concentrations declined slowly in parr recovering in fresh water from crowding stress. In parr previously crowded for 24 and 96 hours, cortisol concentrations were significantly elevated after 24 hours of recovery (ANOVA). In the 96-hour group, cortisol was still significantly elevated after 72 hours of recovery in fresh water. In contrast, relative cortisol concentrations in parr during recovery in salt water were not significantly greater than control concentrations after 24, 48, and 72 hours of recovery, regardless of the duration of previous crowding.

As in parr, relative plasma cortisol concentrations declined more rapidly in those transferred to sea water after crowding than in those transferred to fresh water (Figs. 2.10 and 2.11). Cortisol concentrations in smolts after 24 hours of recovery in fresh water were significantly elevated in fish previously crowded for 8 and 96 hours; after 48 hours of recovery, concentrations were significantly elevated in fish crowded for 8, 24, and 96 hours: and even after 72 hours of recovery, concentrations were normally elevated in **all** test groups, but were significantly elevated only in fish crowded for 8 hours. Cortisol concentrations in smolts recovering in salt water were not significantly elevated after 24 hours of recovery.

Plasma Glucose Concentrations. Relative plasma glucose concentrations declined more rapidly in parr transferred to sea water after crowding than in parr transferred to fresh water (Figs. 2.12, 2.13). Concentrations in parr recovering in fresh water were significantly elevated after 24 hours of recovery for fish crowded at 64 g/liter for 8, 24, and 96 hours; after 48 hours of recovery for fish crowded 24 and 96 hours: and after 72 hours of recovery, concentrations were elevated in all groups, although only concentrations in fish crowded for 96 hours were significantly elevated. In parr

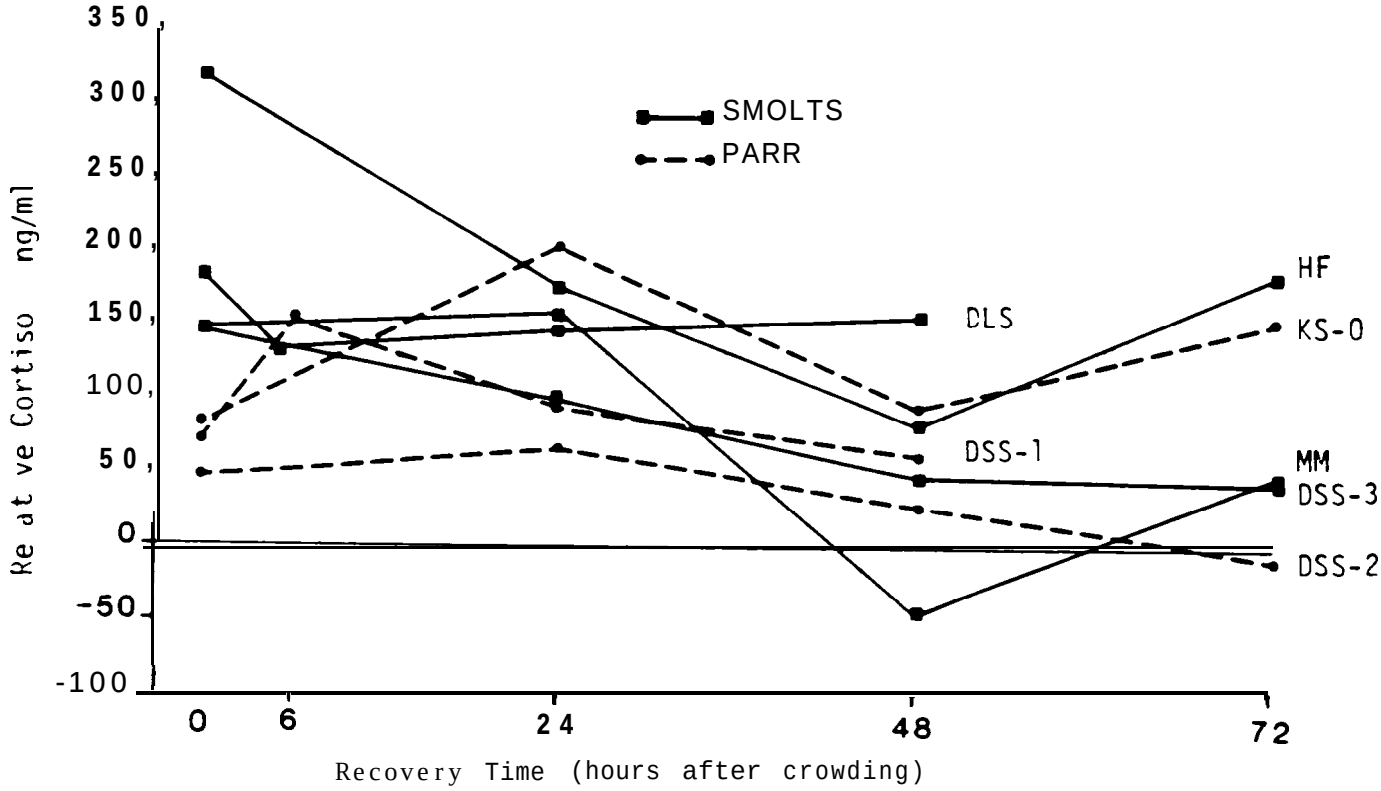


Figure 2.10. Relative plasma cortisol concentrations in seven groups of chinook salmon after 6 to 72 hours of recovery in fresh water from crowding (64 g/liter) for 96 hours. A concentration of zero represents the control: negative relative concentrations indicate the degree to which test were lower than control concentrations. Points represent the mean of one to three pooled, five-fish replicate samples. See Table 2.1 for description of groups of fish.

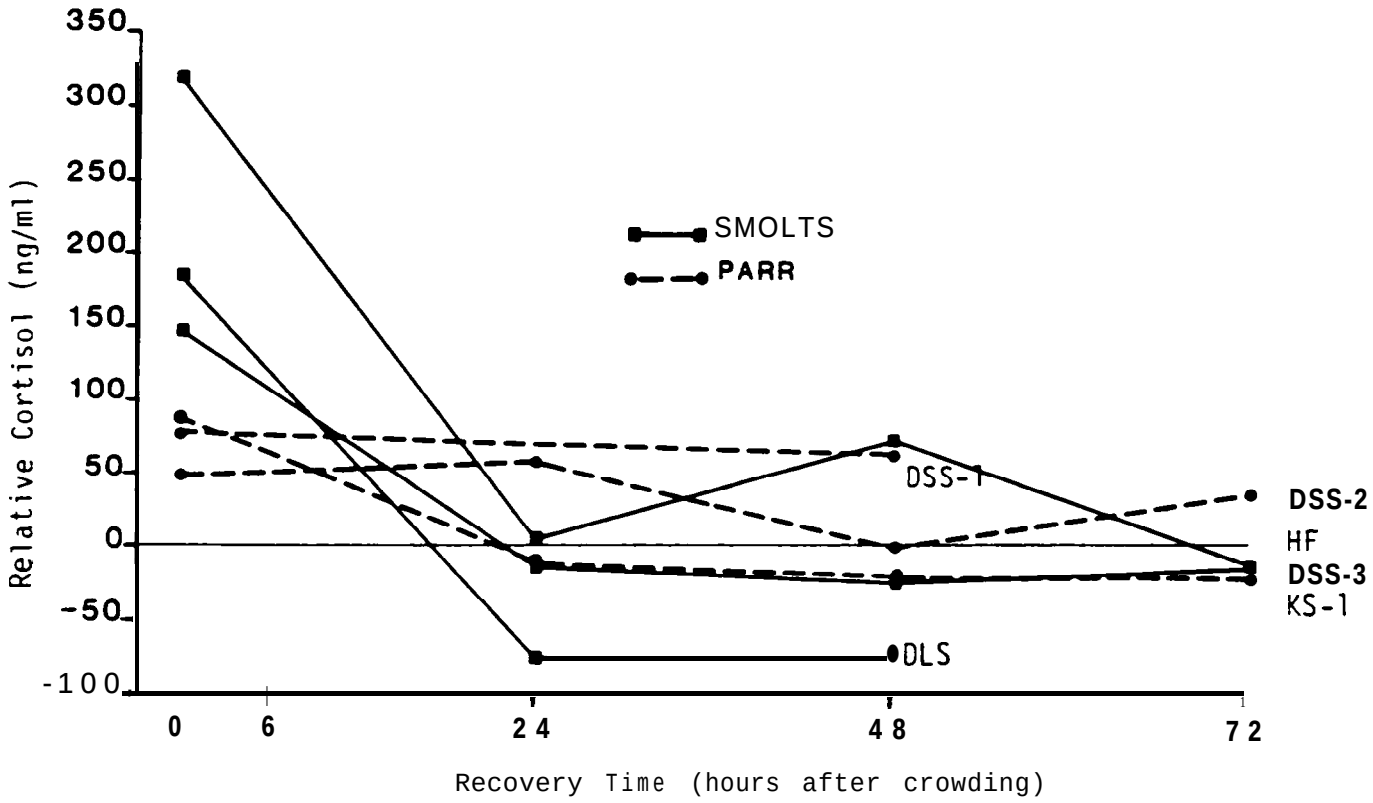


Figure 2.11. Relative plasma cortisol concentrations in six groups of chinook salmon after 6 to 72 hours of recovery in 20°/oo salt water from crowding (64 g/liter) for 96 hours. A concentration of zero represents the control: negative relative concentrations indicate the degree to which test were lower than control concentrations. Points represent the mean of one to four pooled, five-fish replicate samples. See Table 2.1 for description of groups of fish.

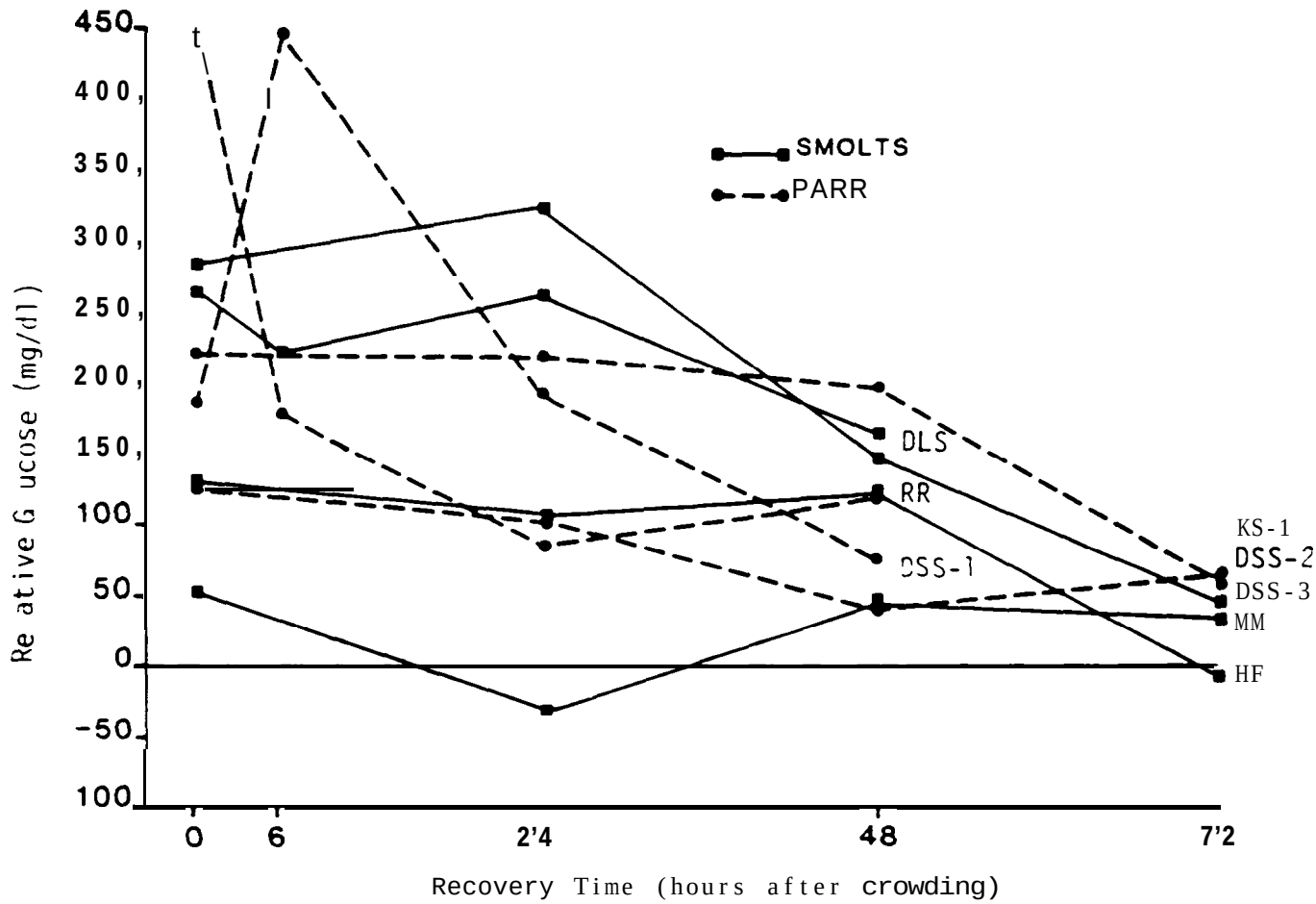


Figure 2.12. Relative (absolute minus control) plasma glucose concentrations in eight groups of chinook salmon after 6 to 72 hours recovery in fresh water from crowding (64 g/liter) for 96 hours. A concentration of zero represents the control: negative relative concentrations indicate the degree to which test were lower than control concentrations. Points represent the mean of one to three Fooled, five-fish replicate samples. See Table 2.1 for description of groups of fish.

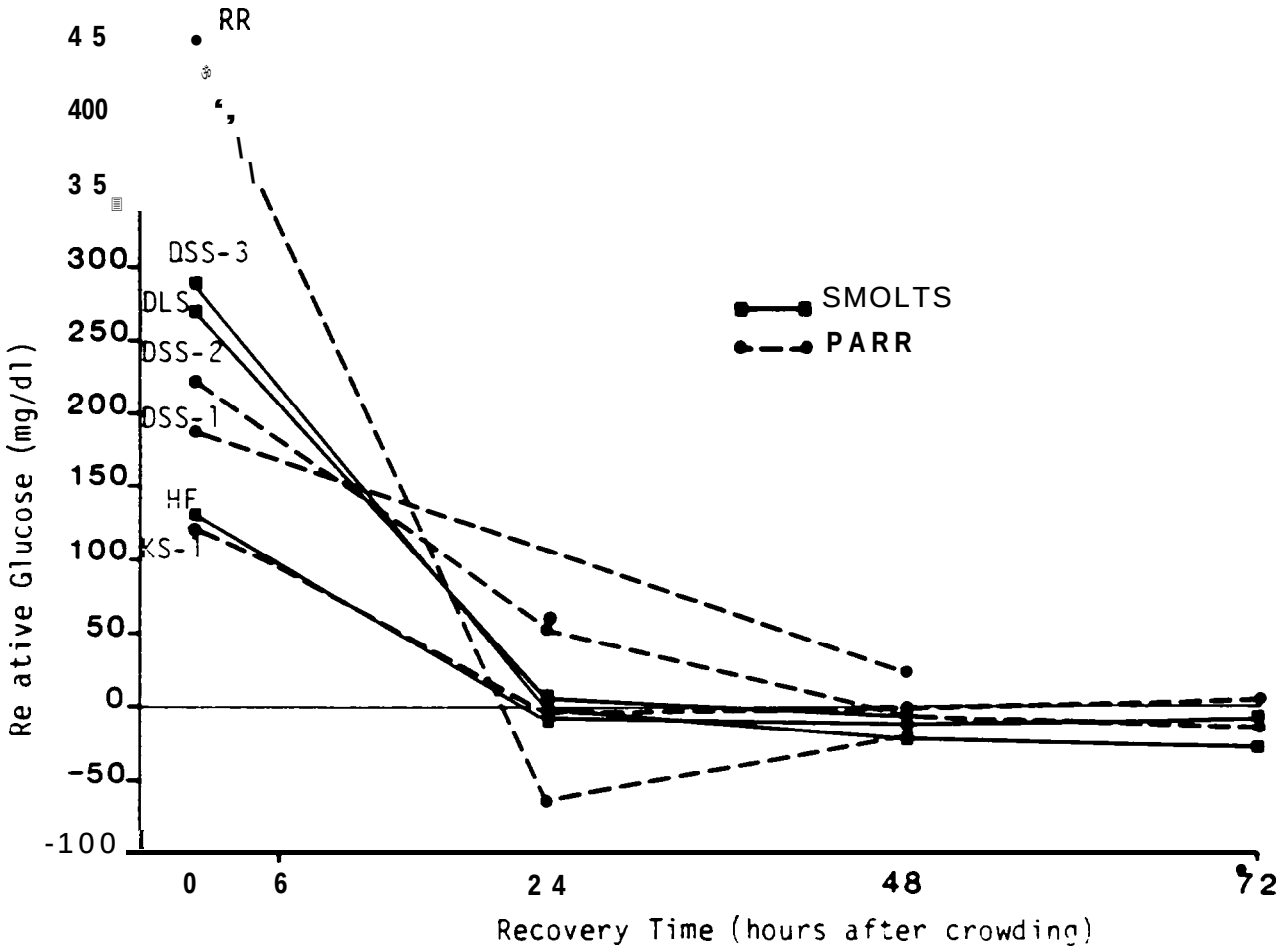


Figure 2.13. Relative (absolute minus control) plasma glucose concentrations in seven groups of chinook salmon after 6 to 72 hours of recovery in 20°/oo salt water from crowding (64 g/liter) for 96 hours. A concentration of zero represents the control: negative relative concentrations indicate the degree to which test were lower than control concentrations. Points represent the mean of one to four pooled, five-fish replicate samples. See Table 2.1 for description of groups of fish.

recovering in sea water, glucose concentrations declined rapidly: after 24 hours of recovery, they were significantly elevated only in fish previously crowded 24 hours.

Differences between rates of recovery in fresh water and salt water were even more pronounced in smolts than in parr. Whereas glucose concentrations were significantly higher than control concentrations in all test groups after 24 hours of recovery in fresh water, none were higher than control concentrations after 24 hours in salt water.

Plasma Na^+ Concentrations. Recovery of plasma Na^+ concentrations was slow, erratic and incomplete in fresh water for both parr and smolts. In contrast, recovery was rapid in salt water: plasma Na^+ concentrations after 24, 48, and 72 hours of recovery were higher than control concentrations. Additional information on accelerated recovery from handling stress after transfer of fish to sea water is given in section 7 of this report.

Changes of Stress Indices in Anesthetized Fish

The slope of a linear regression of plasma cortisol concentration against anesthesia time was non-significant ($P = 0.52$, $n = 76$). but was significant for glucose ($P = 0.0025$, $n = 88$) and for Na^+ ($P = 0.15$, $n = 84$) regressions:

glucose conc. (mg/lOO ml) = 81.0 + 0.58 (time in minutes).

Na^+ conc. (meg/liter) = 158.0 - 0.1 (time in minutes).

Because fish were usually anesthetized for less than 5 minutes before sampling, changes in glucose and Na^+ concentrations would have been negligible.

Discussion

In this study, plasma cortisol concentrations in undisturbed (control) chinook salmon ranged, for the most part, from 19 to 98 ng/ml. Exceptions were the relatively high cortisol concentrations in smolts transported from Lower Granite Dam (which apparently never fully recovered from collection and transportation), and the very low concentrations in Dworshak "small" chinook sampled in March (the most "parr-like" of all the spring chinook salmon tested). Very low concentrations of plasma cortisol in chinook salmon parr have since been confirmed for spring chinook salmon (Little White Salmon and Rapid River stocks) sampled at Dworshak and Kooskia NFHs in the fall and winter of 1982. Plasma cortisol concentrations in these fish ranged from non-detectable to 13 ng/ml from October 1982 through March 1983, and then rose to 30-40 ng/ml in mid April. Specker (1982) reported a similar spring rise in plasma cortisol concentrations in smolting coho salmon. Cortisol influences water and ion movement in the gills, intestine and urinary bladder of fish (see review by Fulmar and Dickhoff 1980), so may play a major role in smoltification and adaptation to sea water.

Plasma cortisol increased above control concentrations within two hours after fish were transferred to crowding cages. Little additional change occurred after 8, 24, and 48 hours, but a significant secondary increase was evident after 96 hours of crowding. The initial increase (of less than 100 ng/ml in all cases but one) was a response to capture and handling. Confinement and crowding did not cause an additional increase in cortisol during the first 48 hours, but no compensation (recovery of cortisol concentrations toward control concentrations) occurred, even in the least densely loaded groups. The secondary increase at 96 hours duration was clearly related to loading density

and was particularly evident in smolts. Physiological mechanisms leading to the delayed secondary increase have not been investigated.

Plasma cortisol concentration increases were higher in crowded smolts than in crowded parr at each of the three loading densities tested. In addition, increased loading density had a greater effect on cortisol in smolts than in parr. These data are in agreement with observations by fish culturists that mortality rates after handling or crowding are much higher in smolts than in parr. Even after smolts have begun to migrate, the physiological response to stressful conditions may continue to increase in intensity. Cortisol concentrations in spring chinook smolts sampled at Lower Granite Dam varied from date to date, and were higher in late April than in mid April (section 3 of this report; Congleton et al. 1984).

Plasma glucose concentrations were similar (64-88 mg/lOO ml) in undisturbed spring chinook salmon parr and smolts. This range is in agreement with that reported in the literature for resting salmonid fishes (Wedemeyer 1972, 1976; Specker and Schreck 1980). Because plasma cortisol concentrations differed in parr and smolts at all loading densities and durations, similar differences were expected in plasma glucose concentrations. However, a significant difference was apparent only at the highest loading density and longest durations tested (128 g/liter for 48 and 96 hours).

Plasma glucose concentrations in crowded parr and smolts rose steadily over time, and did not plateau between 2 and 48 hours as did cortisol concentrations. Because elevated plasma glucose is a secondary, metabolic response to a primary elevation of cortisol (and adrenaline), glucose

concentration changes lag behind cortisol concentration changes following exposure to a stressor.

Plasma Na^+ concentrations responded to the initiation of crowding more slowly than plasma cortisol and glucose concentrations. Increases in branchial perfusion and in permeability to water and ions (due primarily to the effects of adrenalin) resulted in a significant plasma Na^+ decline after 24 hours of crowding. At the two higher loadings (64 and 128 g/liter), Na^+ concentrations declined to a greater extent in smolts than in parr, possibly because the physiological transition from hyperosmotic to hypoosmotic regulation had already begun in the smolts. Among many other changes, smoltification involves a decline in glomerular filtration rate (Holmes and Strainer 1966), thereby decreasing the extent to which diuresis can compensate for increased branchial water influx. In addition, the number of branchial cells responsible for Na^+ uptake may decline as the number of cells responsible for ion excretion (chloride cells) increases (T. Bradley, Dworshak NFH, personal communication).

Plasma cortisol, glucose and Na^+ concentrations recovered to baseline control concentrations more rapidly in parr and smolts transferred to 20°/oo sea water after crowding than in those transferred to fresh water. Recovery in sea water was usually complete in 24 hours: recovery in fresh water was often incomplete after 72 hours. The prolonged depression of plasma Na^+ in fresh water after crowding indicated that branchial Na^+ uptake could not quickly restore blood Na to the pre-crowding concentration. Restoration of plasma Na may have been especially slow due to low ambient Na^+ concentrations (about 1 ppm) in the hatchery water supply. In contrast, the steep Na^+ gradient from sea water to blood caused a rapid increase in blood Na^+, which stabilized at a higher concentration in

lgrecoveryl' fish in 20°/oo sea water than in control fish held in fresh water.

The rapid decline in cortisol and glucose concentrations in fish transferred to sea water demonstrated a linkage between osmotic and ionic homeostasis and these indices. One of the major effects of elevated cortisol during stress is to bring about gluconeogenic mobilization of energy reserves. Restoration of plasma Na+ by transfer to sea water would eliminate the need for Na+ uptake by branchial ion exchange, an energetically costly process, allowing plasma cortisol concentrations to decline more rapidly than possible in fresh water (see section 7 of this report).

Summary

1. Cortisol was undetectable in plasma of spring chinook salmon sampled in March, but ranged between 19 and 98 ng/ml in plasma of the same and three other groups of spring chinook salmon sampled in April, May and June. These and other cited data indicate that plasma cortisol concentrations rise in smolting salmon.

2. Plasma cortisol and glucose concentrations were significantly above control concentrations 2 hours after initiation of crowding and in all subsequent samples. Plasma Na+ was not significantly below control concentrations at 2 and 8 hours, but was significantly depressed at 24 to 96 hours.

3. Plasma cortisol concentrations were higher in crowded smolts than in crowded parr at each of the three loading densities tested. In addition, glucose concentrations were higher and Na+ concentrations were lower in smolts than in parr, although these

differences were apparent only at the two higher loading densities and longer durations (48 and 96 hours).

4. Plasma cortisol, glucose and Na^+ concentrations returned toward control concentrations more rapidly in parr and smolts transferred to sea water after crowding than in parr and smolts transferred to fresh water. Recovery in sea water was usually complete in 24 hours; recovery in fresh water often was not complete at 72 hours.

5. Concentrations of cortisol, glucose and Na^+ in the plasma of anesthetized fish changed slowly or not at all.

3. CHANGES IN STRESS INDICES ASSOCIATED WITH EACH STEP IN COLLECTION AND TRANSPORTATION PROCESS

Downstream-migrating smolts of chinook salmon and steelhead trout entering turbine intakes at Lower Granite Dam are diverted upward into gatewells by traveling screens (30%-50% of the fish entering the intakes are not successfully diverted and pass through the turbines). Fish then pass through orifices (two per gatewell, each 20 cm in diameter) into a collection flume, which carries them to the upper end of a 1.07-m diameter pipe (Fig. 3.1). This pipe drops 18 m to the fish handling and loading facility. Flow velocities in the bypass pipe approach 9 m/second and flow is totally turbulent. Deceleration of flow in a stilling basin at the terminus of the bypass pipe also produces strong turbulence. The fish pass over a dewatering plate below the stilling basin and through the submerged bars of a trash separator, enter a short, open flume, and fall into a raceway. Fish are held in raceways for various periods ranging from a few hours to 2 days before they are loaded onto fish transport trucks or barges and taken to the upper Columbia estuary below the lowermost dam on the Columbia River.

Several of the events experienced by fish during collection and transportation are potentially traumatic, either physically or psychologically. Experimental work with salmonid fishes has shown that a variety of traumatic experiences can elicit a non-specific stress response similar to that described for other vertebrate animals (Wedemeyer 1976; Strange et al. 1978; Barton et al. 1980). The secondary effects of the stress response in fishes include disturbances of metabolic and osmoregulatory homeostasis and of immune responsiveness (Strange et al. 1977; Pickering and Duston 1983). These secondary effects

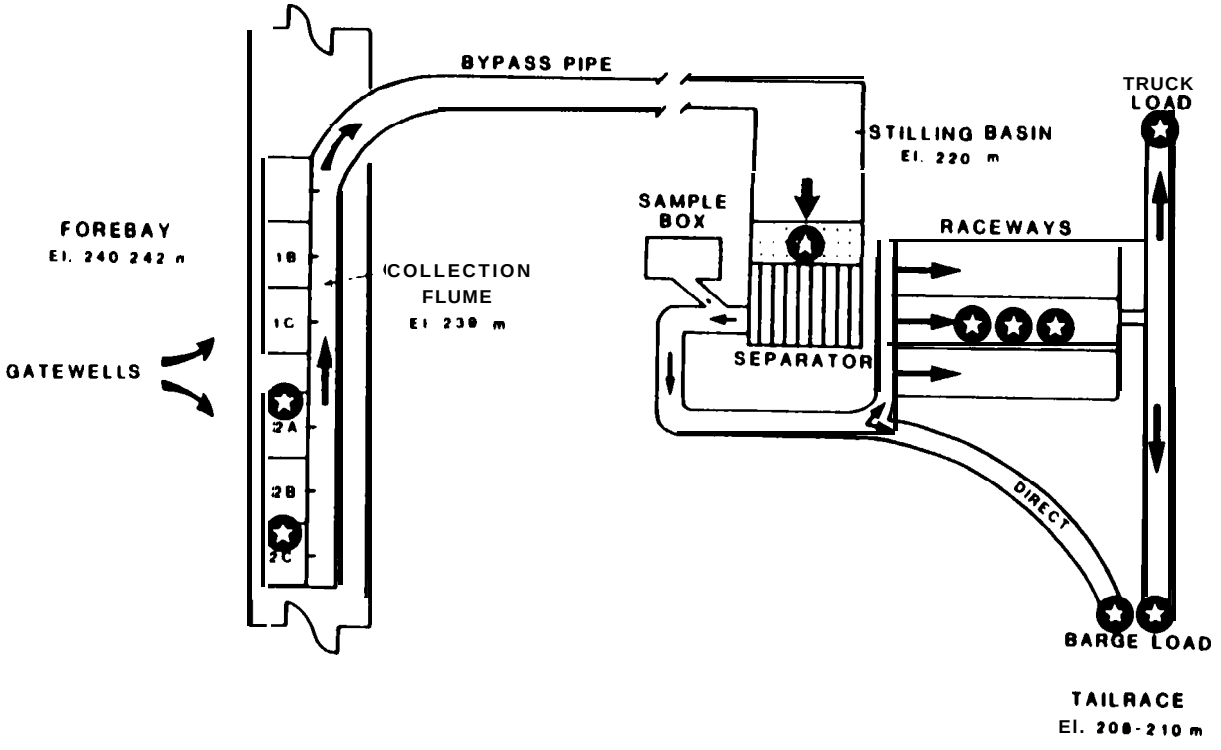


Figure 3.1. Schematic of collection facility for downstream migrants at Lower Granite Dam. Sampling points are indicated by stars.

could cause delayed mortality of collected and transported fish after release in the estuary.

To identify stressful steps in the collection and transportation process, we collected blood samples from chinook salmon at various points in the process and analyzed the samples for three stress response indices--plasma cortisol, glucose, and Na^+.

Methods and Materials

Chinook salmon were sampled at eight points in the collection and transportation procedure at Lower Granite Dam on four dates in 1982: April 14-15, 16-17, 21-22, and 27-28. Migrating steelhead smolts were sampled on April 29-30, and fall chinook salmon were sampled on June 6-7.

The first samples on each sampling date were usually taken at or shortly after sunset from the bulkhead gatewell A- and C-slots of turbine no. 1 or no. 2 (exceptions were on April 14, when gatewell 6A and 6C were sampled, and April 16, when gatewells 1A and 5C were sampled). A second sample was taken of fish passing over the perforated plate just upstream from the wet separator at the terminus of the bypass pipe (post-bypass sample; see Fig. 3.1). Next, a movable crowder was used to clear fish from the upstream end of one raceway. Fish leaving the separator were collected in this section of raceway for 45 minutes and then sampled (2000-2103 hours). After withdrawing the crowder, raceway samples were taken the following morning at about 0400 to 0600 hours (6-8 hours after the fish entered the raceway, on the average) and 0800-1000 hours (10- 12 hours after the fish entered the raceway). Fish were loaded from the raceways into a truck (April 14-15 and 16-17 samples) or barge (April 21-22 and 27-28 samples) between 1000 and 1200 hours, and a sample was taken 30-45 minutes after loading

was begun. Finally, samples were taken at Bonneville Hatchery of fish arriving by truck (after an 8-hour trip) or barge (after a 38- to 40-hour trip).

We used a dip basket of the type described by Bentley and Raymond (1968) to take gatewell samples. Because large numbers of fish were captured with this device, a subsample was taken from the dip basket with a dip net. We collected other samples (from raceways, trucks, and barges) with a rectangular frame net, 30 cm x 60 cm, that was lowered to the bottom and then rapidly hauled to the surface. All nets were fitted with resenroirs so that sampled fish were never removed from the water.

Procedures followed for blood sampling and for cortisol, glucose and electrolyte analyses were described in section 2. The standard sample size was 15 fish: blood was pooled from each 5-fish subsample. In addition, we also determined lactic acid concentrations for plasma samples from some spring chinook salmon collected at Lower Granite Dam (Sigma Chemical lactate analysis kit 826-W).

Results

Changes in Stress Indices of Spring Chinook Salmon During Collection and Transportation

Plasma cortisol levels in spring chinook salmon sampled from gatewells at Lower Granite Dam in April 1982 averaged 76 ng/ml (four sampling dates, three subsamples of five to six fish each from both an A-slot and a C-slot gatewell on each date). Mean cortisol concentrations were consistently higher in samples from all sampling points taken on April 27-28 than in similar samples taken on other dates (Fig. 3.2). Disregarding the April 27-28 data, cortisol concentrations averaged 41 ng/ml (95% CI = 28-54 ng/ml) in

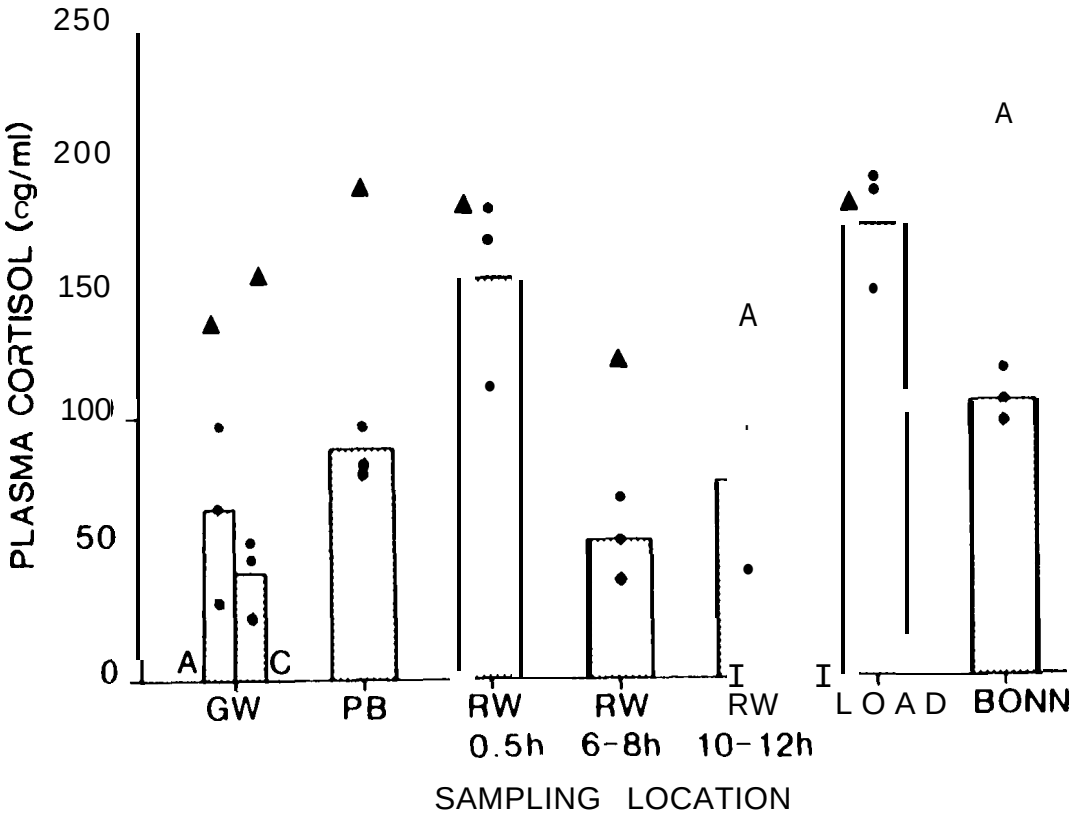


Figure 3.2. Mean plasma cortisol concentrations in chinook salmon sampled during collection and transportation from Lower Granite Dam to below Bonneville Dam. Vertical bars indicate overall mean concentrations in fish sampled on April 14-15, 16-17, and 21-22, 1982; circles indicate mean concentrations in fish sampled on each date. Triangles indicate mean concentrations in fish sampled on April 27-28, 1982. Sampling points were gatewells (GW), A- and C-slots, post-bypass (PB), raceways (RW) after indicated intervals of time, after loading on barges (LOAD), and after arrival at Bonneville Dam in barges (BONN).

chinook salmon sampled from C-slot gatewells and 64 ng/ml (95% CI = 29-93 ng/ml) in chinook salmon sampled from A-slot gatewells. Cortisol levels rose in samples taken below the bypass pipe ($\bar{x}$ = 112 ng/ml for all dates) and in samples taken 45 minutes after fish had entered a raceway ($\bar{x}$ = 160 ng/ml). During the first 6-8 hours of nighttime raceway confinement, plasma cortisol fell to 69 ng/ml (mean for all dates) and remained relatively low (88 ng/ml) through the mid-morning (0800-1000 hours) sample. After fish were removed from the raceways and loaded onto a truck or barge (1000-1200 hours), plasma cortisol levels rose to 176 ng/ml (mean for all dates). Cortisol levels were similar after loading onto trucks (146, 191 ng/ml) and onto barges (180, 189 ng/ml) .

Plasma cortisol was much higher (249 ng/ml) in the single group of chinook salmon sampled at Bonneville after truck transport (April 17) than in three of the four groups arriving by barge (sample means of 99, 107, and 117 ng/ml; arrival dates April 24, 26, and 28, respectively). The fourth group of barged fish (arrival date April 30) had a relatively high mean plasma cortisol concentration of 214 ng/ml.

Glucose levels were no higher in post-bypass and head-of-raceway spring chinook salmon samples than in gatewell samples (overall range of means 102-112 mg/lOO ml for all dates; Fig. 3.3). The first increase was seen in fish sampled after 6-8 hours of raceway residence ($\bar{x}$ = 146 mg/lOO ml for all dates); glucose concentrations were similar in mid-morning raceway and post-loading samples. The increase in glucose concentrations (gatewell, post-bypass, and initial raceway sample means versus subsequent sample means) was significant ($P < 0.01$; Mann-Whitney U test). A further increase in plasma glucose (to 170 mg/lOO ml) was seen in the one group of chinook salmon sampled after transportation

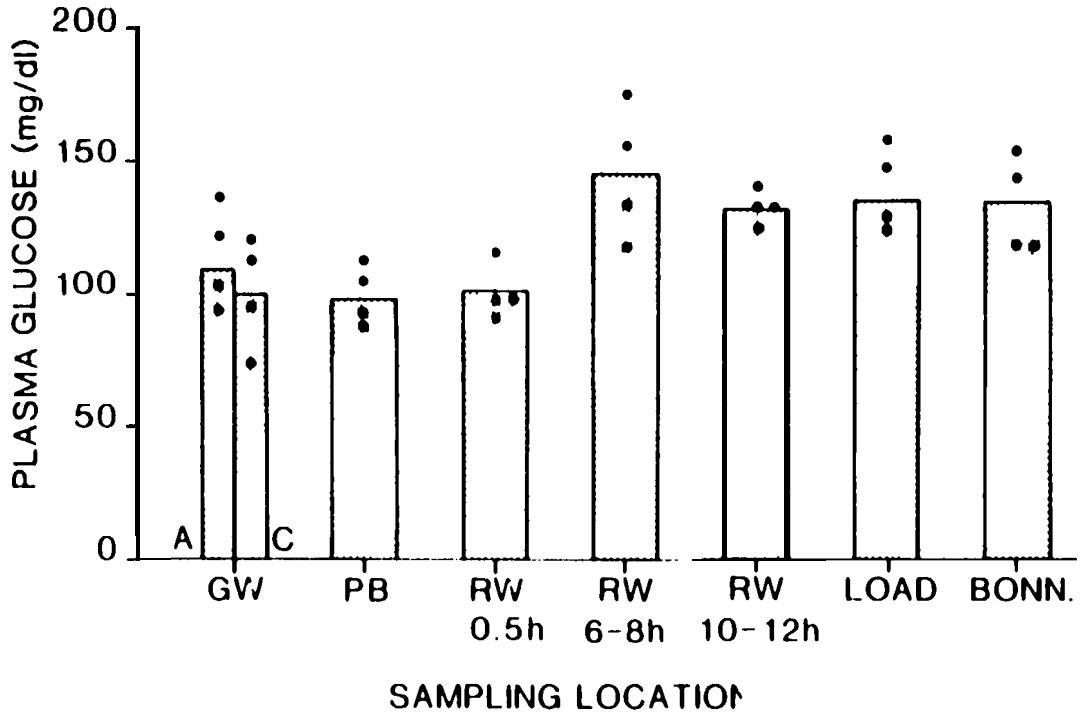


Figure 3.3 Mean plasma glucose concentrations in chinook salmon sampled during collection and transportation from Lower Granite Dam to below Bonneville Dam. Vertical bars indicate overall mean concentrations in fish samples on all dates (April 14-15, 16-17, 21-22, and 27-28, 1982); circles indicate mean concentrations in fish samples on each date. Sampling points were as given for Figure 3.1.

to Bonneville Dam by truck (April 17); glucose levels in the four groups of barged fish were somewhat lower (118, 118, 152, 143 mg/lOO ml on April 24, 26, 28, and 30, respectively).

We determined plasma lactate concentrations in spring chinook salmon collected at the standard sampling locations on April 16-17, 1982. Concentrations increased from the gatewell to the initial raceway sample, reaching a peak value of 67 mg/lOO ml, and then declined (Fig. 3.4).

In the series of samples taken at the collection facility on April 21-22, plasma Na+ declined progressively from 166-179 meg/liter in gatewell samples to 140 meq/liter in post-loading samples (Fig. 3.5), and declined further to 123 meg/liter during barge transportation to Bonneville Dam. Plasma Na+ levels for other groups of barged and trucked fish arriving at Bonneville were also low (122-131 meg/liter).

Plasma Ca++ also declined in concert with Na+ in the April 21-22 sample series, averaging 4.4 - 4.7 meg/liter in fish from gatewell samples, and 3.2 meg/liter after their arrival at Bonneville.

Plasma Na+ concentrations in fish sampled at the standard sampling locations on April 14-15 declined much less than Na+ concentrations on April 21-22. The post-loading Na+ level (144 meg/liter) was similar to that on April 21-22, but gatewell Na+ levels were considerably lower (145-159 on April 14 versus 166-179 meg/liter on April 21). Ca++ did not decline discernibly during collection and transportation on April 14-15, fluctuating between 3.0 and 4.7 meg/liter.

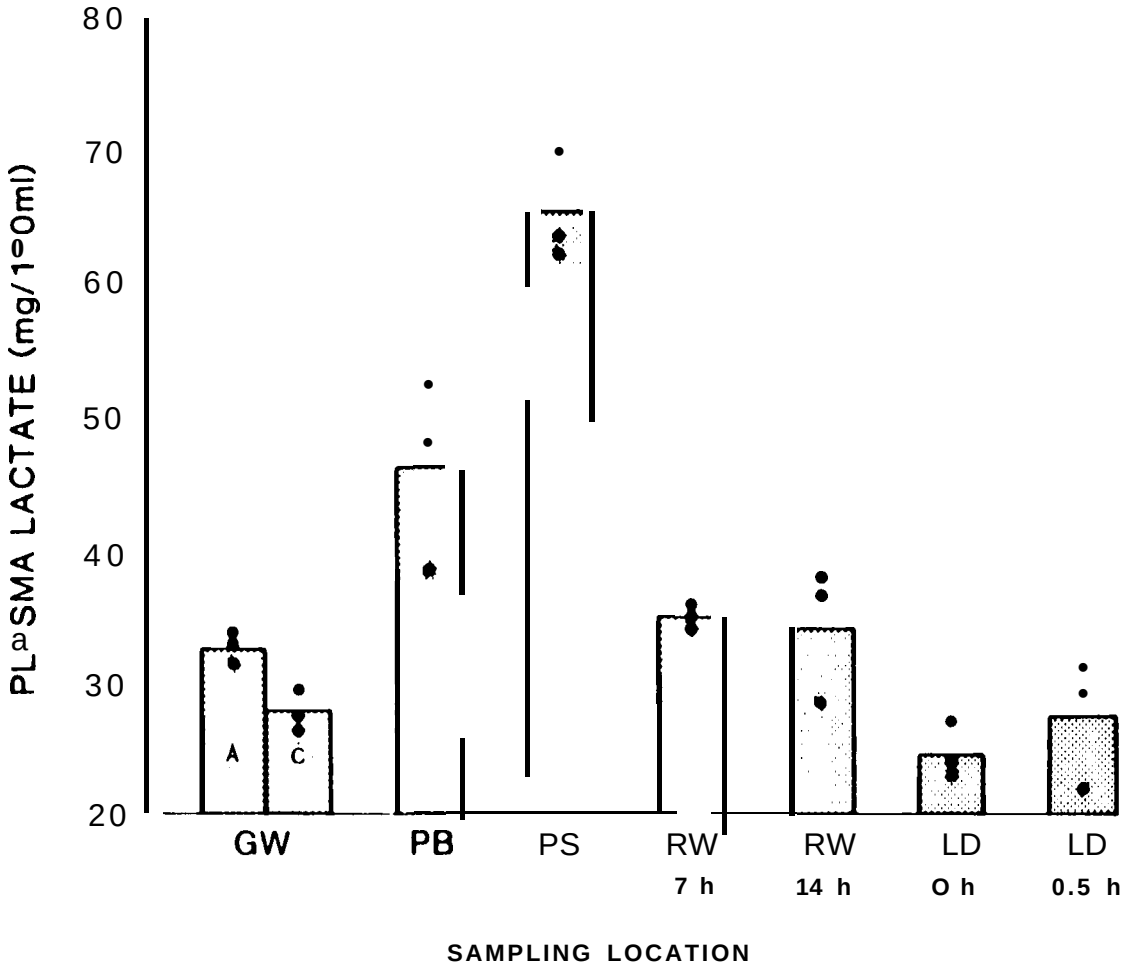


Figure 3.4. Mean plasma lactate ion concentrations in chinook salmon sampled in the collection system at Lower Granite Dam on April 16-17, 1982. Vertical bars indicate mean concentrations; circles indicate individual subsample concentrations (five fish each). Sampling points were gatewells (GW), A- and C-slots, post-bypass (PB), raceways (RW) after indicated periods of time, and after loading on truck (LD).

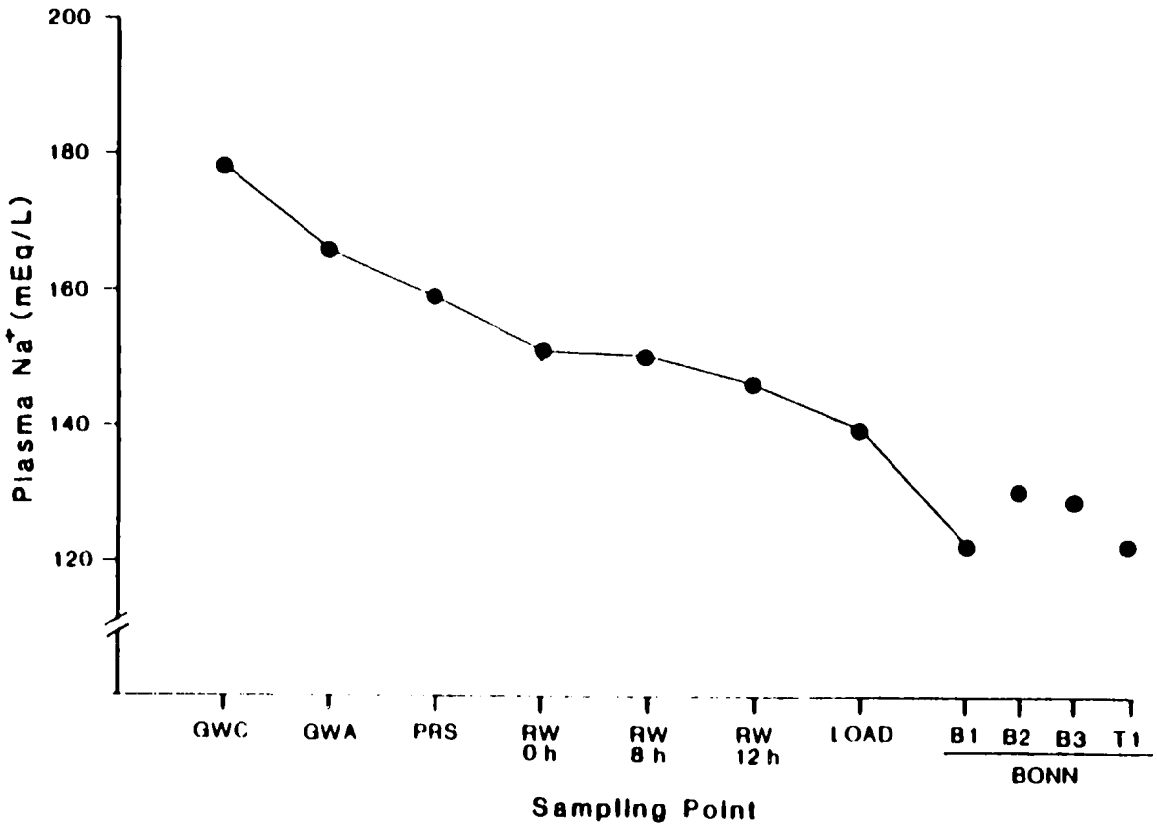


Figure 3.5. Plasma Na^+ concentrations for spring chinook smolts sampled during collection and transportation from Lower Granite Dam (April 21-24, 1982). Sample locations were as follows: GWC, gatewell 2C; GWA, gatewell 1A; PRS, preseparator; RW, raceway (after indicated time intervals); LOAD, after loading on barge; B1, barge after arrival at Bonneville. Also shown are plasma Na^+ concentrations for chinook arriving at Bonneville by barge on April 26 (B2) and April 28 (B3) and by truck on April 17 (T1).

Recovery of Transported Fish at Bonneville

Spring chinook salmon smolts transported to Bonneville by truck on April 17, 1982, were sampled at arrival with a reservoir net and transferred to 120-liter cans. The fish were then driven to the nearby National Marine Fisheries Service (NMFS) wet laboratory and poured into tanks 1.8 m in diameter supplied with circulating Columbia River water. Samples were taken for analysis of plasma cortisol, glucose, and Na^+ after tag fish were loaded at Lower Granite Dam, after arrival at Bonneville, and after 12, 24, 48, 72, 96, and 120 hours of recovery. Several samples were also taken from other groups of transported fish after various periods of recovery. This work was carried out in conjunction with NMFS personnel also investigating the effects of transportation stress.

Plasma cortisol averaged 41 ng/ml in chinook salmon sampled from the raceways at Lower Granite Dam at 1115 hours on April 17. One hour later, immediately after fish had been loaded onto a fish tanker, cortisol had climbed to 102 ng/ml. It continued to rise, reaching 146 ng/ml after 30 minutes and 249 ng/ml when the truck arrived at Bonneville, 8 hours later (Fig. 3.6). During the recovery period, cortisol fell to a low of 53 ng/ml after 2 days and then increased slightly each succeeding day, reaching 88 ng/ml on the 5th day. Similar cortisol levels were measured in fish transported at other times and sampled after 48, 72, and 144 hours of recovery.

Plasma glucose also rose in response to loading and transportation, peaking at 286 mg/100 ml after 12 hours of recovery (Fig. 3.7). Following this peak, glucose declined to a low of 126 mg/100 ml after 4 days before rising slightly to 156 mg/100 ml on the following (5th) day.

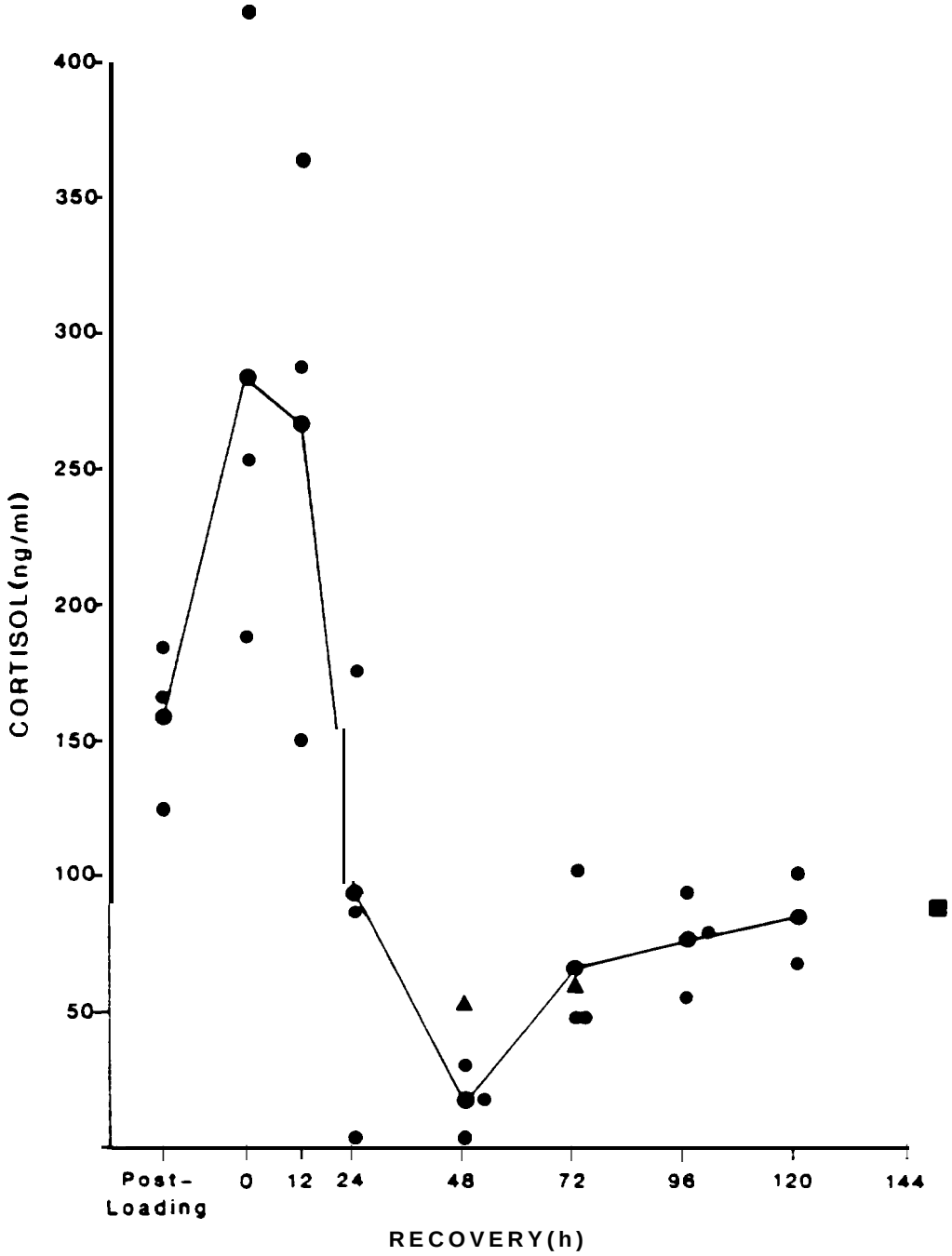


Figure 3.6. Mean plasma cortisol concentrations in spring chinook smolts during a five-day recovery period in fresk water following transportation by truck from Lower Granite Dam to Bonneville Dam (April 17, 1982). Mean (larger circles) and subsample cortisol concentrations (smaller circles! are plotted for each sample (n = 15 to 18). Also shown are mean recovery values for chinook arriving by truck on April 15 (at 48 and 72 hours only; triangles and by truck on April 18 (at 144 hours only; square).

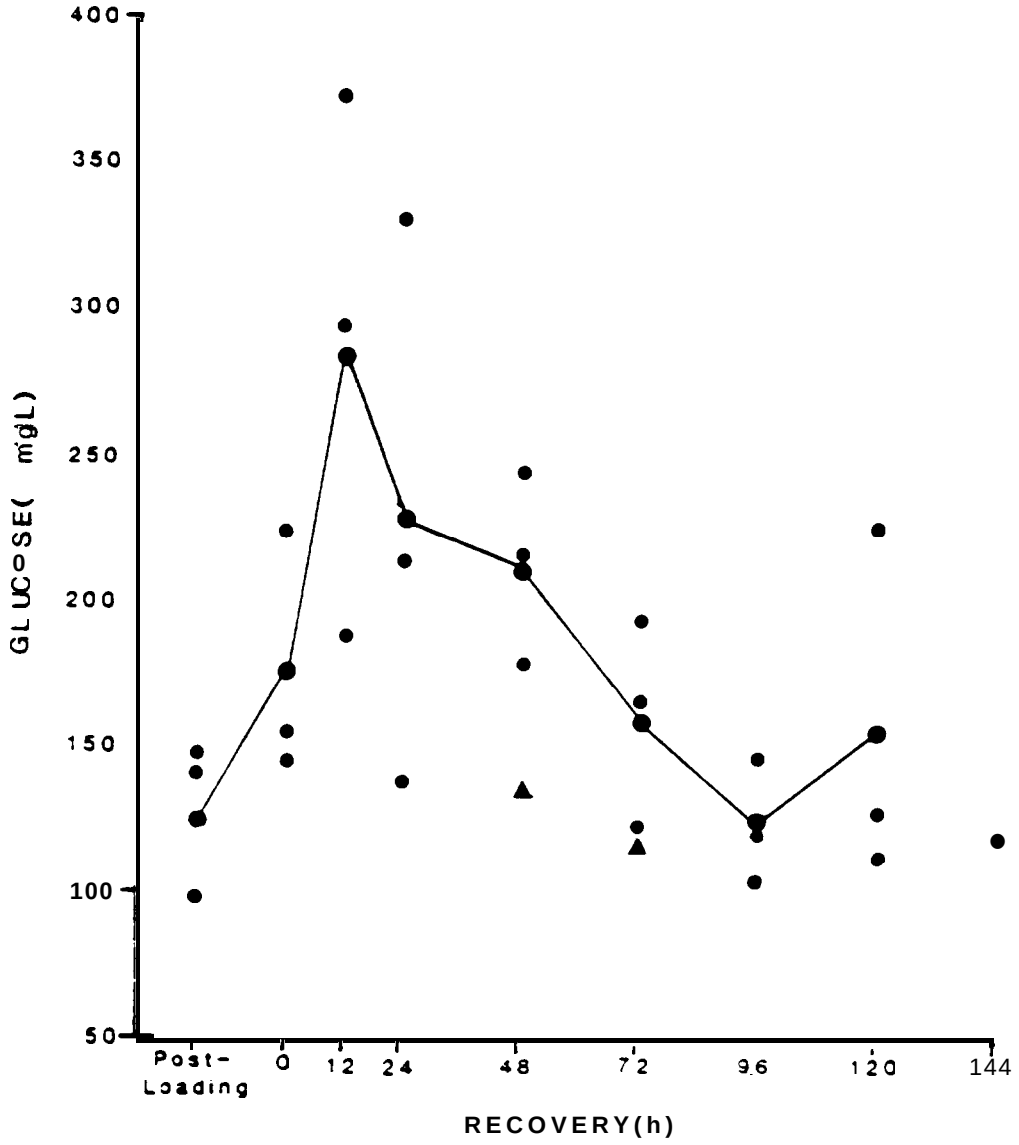


Figure 3.7. Mean plasma glucose concentrations in spring chinook smolts during a five-day recovery period in fresh water following transportation by truck from Lower Granite Dam to Bonneville Dam (April 17, 1982). Mean (larger circles) and subsample glucose concentrations (smaller circles) are plotted for each sample (n = 15 to 18). Also shown are mean recovery values for chinook arriving by truck on April 15 (at 38 and 72 hours only; triangles) and by truck on April 18 (at 144 hours only; square).

Plasma Na+ was depressed (124 meg/liter) in this group of chinook salmon when they arrived at Bonneville, as in other groups sampled after transportation. Recovery of plasma Na+ was steady over the 4-day sampling period, but values had not returned to pre-collection levels (160-180 meg/liter) by the end of this period (Fig. 3.8).

Plasma Na+ was also determined for chinook salmon subjected to 48 hours of saltwater challenge (28°/oo "Marine Environment") and then allowed to recover for various periods in fresh water (NMFS study). Impaired ionoregulatory ability was evident only in the group challenged immediately upon arrival at Bonneville (no recovery); fish permitted 24 hours of recovery or longer regulated plasma Na+ in the range of 170-176 meg/liter (Fig. 3.8).

Die1 Variation in Stress Indices

On April 14-15, chinook salmon smolts were sampled above the separator at 4-hour intervals over a 20-hour period. The objective was to determine whether a die1 cycle could be detected in stress indices of fish entering the facility.

Plasma glucose showed a distinct die1 cycle (Fig. 3.9): mean glucose concentrations were lowest in the late evening and highest at midday. Plasma cortisol followed a similar pattern (Fig. 3.9), except that the lowest mean concentrations occurred earlier in the evening (1900 hours versus 2300 hours for glucose), and a second low value occurred in the 0700-hour sample.

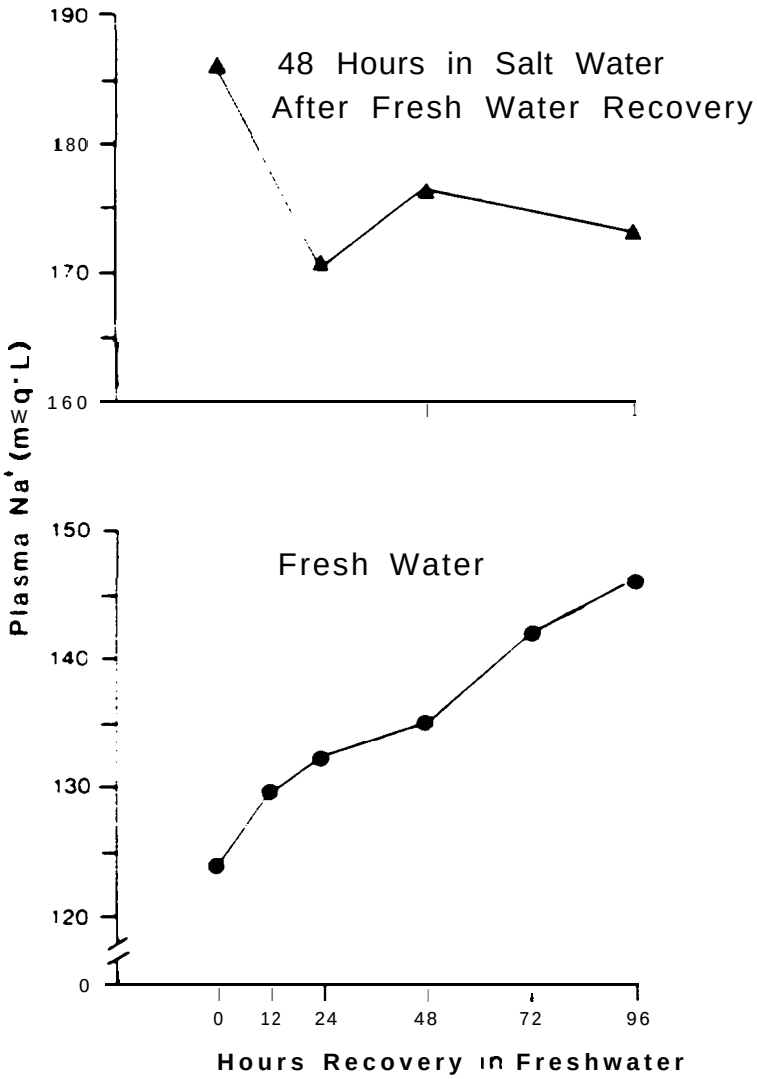


Figure 3.8. (Below) Mean plasma Na+ concentrations in spring chinook salmon transported from Lower Granite Dam to Bonneville Dam by truck ;April 17, 1982) and held in fresh water for 4 days. (Above) Mean plasma Na+ concentrations in spring chinook salmon held in sea water (28°/oo) for 48 hours after various intervals of recovery in fresh water (horizontal scale on lower figure).

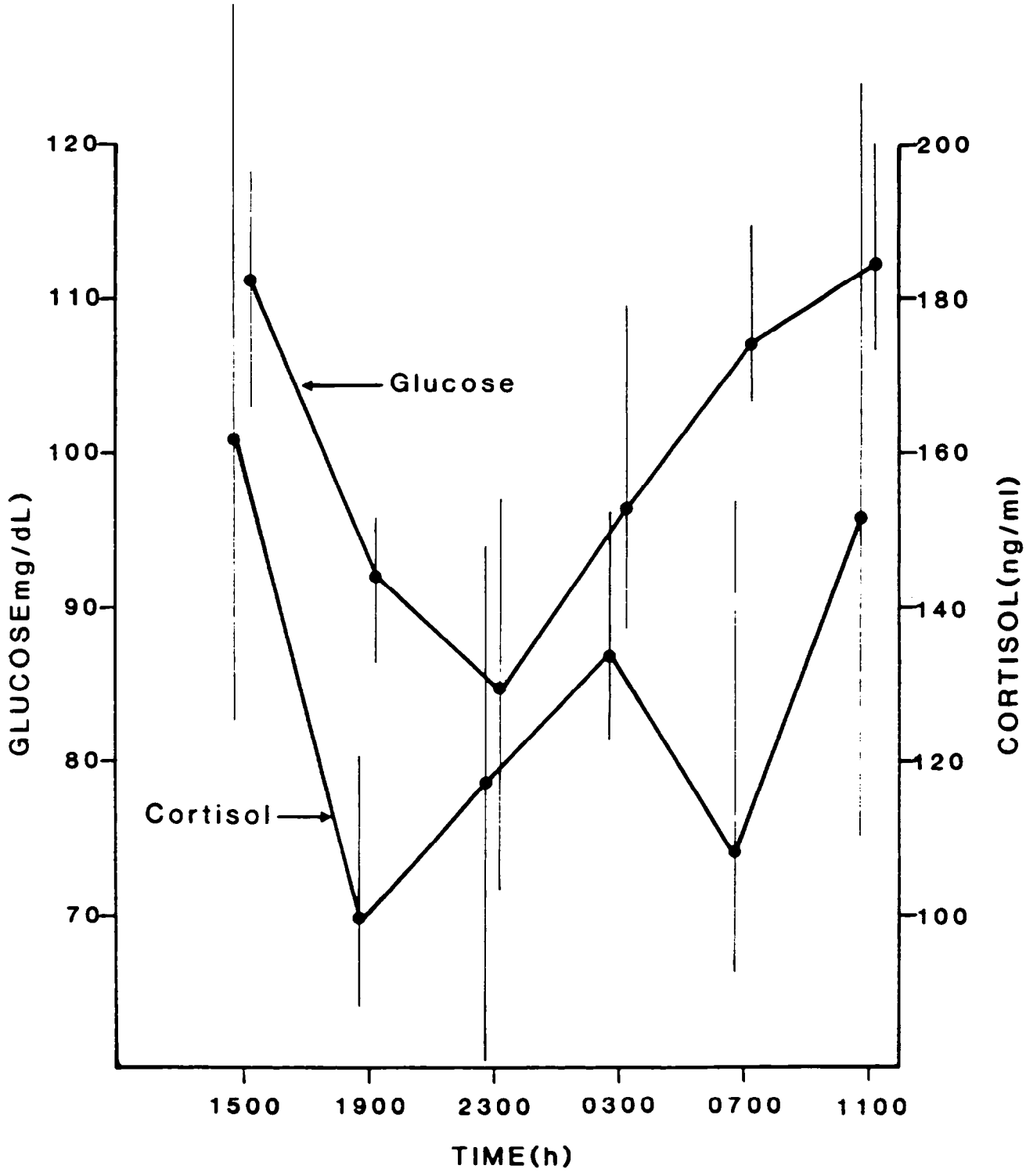


Figure 3.9. Diel variation in plasma cortisol and glucose levels in spring chinook smolts collected above the separator at the Lower Granite fish handling facility (April 14-15, 1982). The mean and range of subsample values is plotted for each sample (n = 15 to 20).

Recovery from Anesthesia and Handling

We sampled one group of spring chinook salmon that was crowded from a raceway into the fish marking building, anesthetized, and handled in a manner simulating the handling received during normal procedures for branding and microwire-tagging (May 5, 1982). The fish did not actually receive a brand or tag. The fish were sampled once before recovery from the anesthesia (50 ppm MS-222) and at later intervals during 84 hours of recovery in an outdoor tank 1.2 m in diameter. The sample size was 10 fish; blood was pooled in 2 subsamples of 5 fish each. Samples were not taken before the fish were crowded into the marking building and anesthetized.

Plasma cortisol rose abruptly to 284 ng/ml during the first hour of recovery (Fig. 3.10). Cortisol declined to 109 ng/ml after 12 hours of recovery and to 83 ng/ml after 36 hours, but increased to 248 ng/ml after 84 hours. Glucose rose to 260 mg/lOO ml during the first hour of recovery, and climbed still further by 12 hours recovery (Fig. 3.10). A subsequent slow decline extended to the last sample at 84 hours.

Changes in Stress Indices for Steelhead Trout and Fall Chinook Salmon

Changes in plasma cortisol and glucose levels in steelhead trout passing through the Lower Granite facility paralleled changes seen in these indices in spring chinook salmon. Cortisol was low in gatewell samples (30 ng/ml), considerably higher in post-bypass samples (98 ng/mU, peaked in the initial raceway sample (160 ng/ml), and subsequently declined. Glucose levels did not rise above 110-130 mg/lOO ml in the gatewell, pre-separator or initial raceway samples, but increased to 170-200 mg/lOO ml after fish had been held in raceways for 6-9 hours.

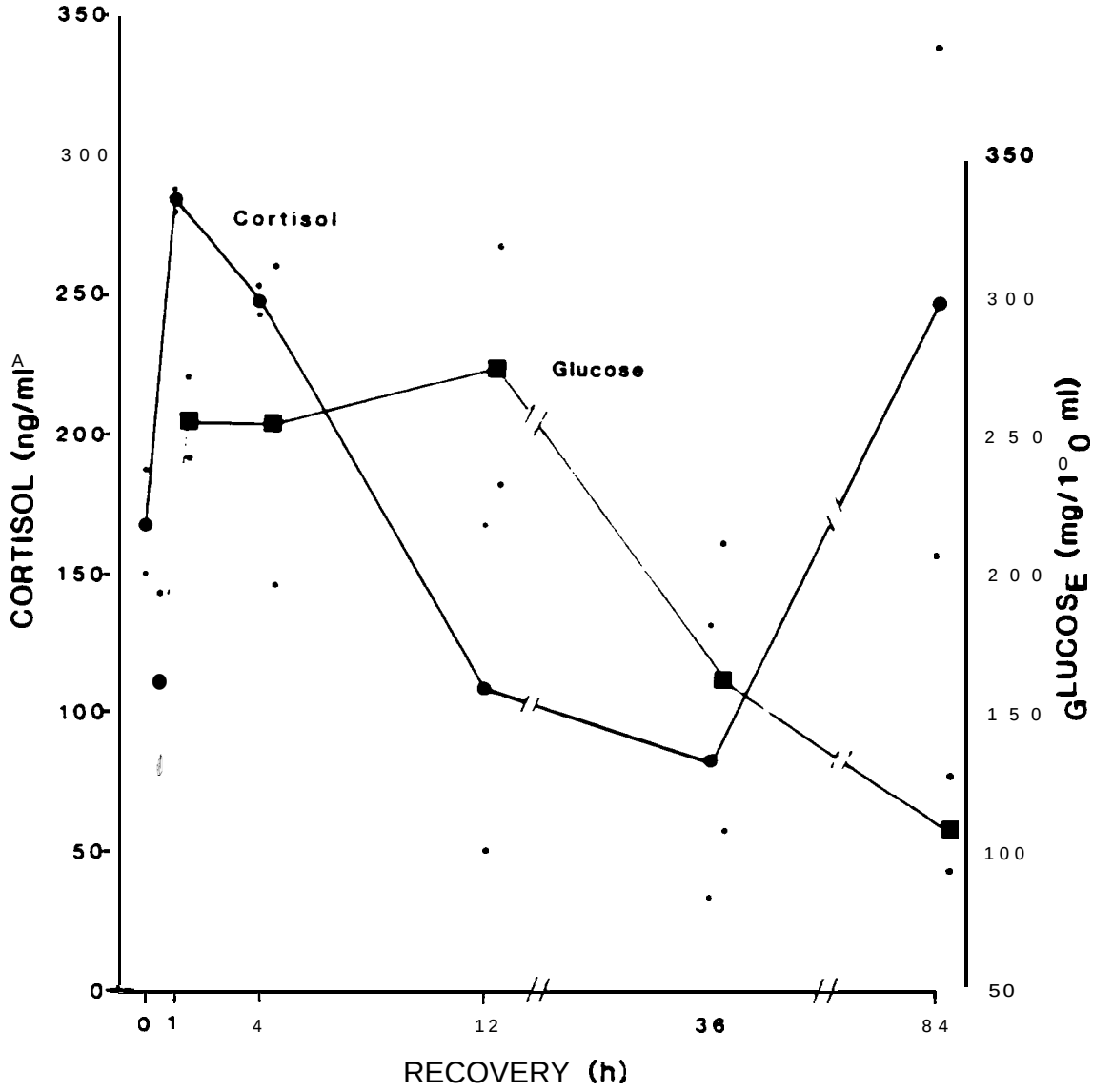


Figure 3.10. Mean plasma cortisol and glucose concentrations in spring chinook salmon recovering from anesthesia and sham marking at Lower Granite Dam. The first sample (0 h) was taken from anesthetized fish immediately after sham marking.

A gatewell sample of fall chinook salmon was not obtained, and complete samples (n = 15) were not obtained at some other sampling points. In addition, blood samples were small due to the small size of the fish, sometimes precluding duplicate analyses. Mean post-bypass and initial raceway cortisol concentrations (50 and 102 ng/ml, respectively) were lower than for spring chinook salmon (and steelhead trout), but in a later sample of fish held in a raceway for 6-8 hours were as high or higher (118 ng/ml) than for spring chinook salmon after similar periods of raceway residence. On the other hand, mean post-bypass and initial raceway glucose concentrations (131 and 147 mg/lOO ml, respectively) were higher than obsenred for spring chinook salmon, but the concentration for the later 6- to 8-hour raceway sample was similar (146 mg/lOO ml) to those observed for spring chinook salmon.

Discussion

Stressful events during collection and transportation are best identified by reference to plasma cortisol levels, which respond more quickly than do plasma glucose or electrolyte levels. The seasonal mean cortisol levels of 40-60 ng/ml measured in gatwell samples at Lower Granite Dam (disregarding the higher cortisol concentrations in fish sampled from gatewells on April 27-28) were moderately higher than baseline levels of 30-40 ng/ml measured in experimental groups of spring chinook salmon studied at Dworshak NFH (except for Dworshak NFH "large" chinook salmon smolts, with a baseline level of 98 ng/ml). Apparently chinook salmon were not highly stressed by being diverted from turbine intakes into gatewells on three of the four dates sampled. On the other hand, the higher mean plasma cortisol concentrations (135, 154 ng/ml) in fish sampled

from gatewells on April 27-28 were well above baseline concentrations.

Elevated plasma cortisol levels in fish exiting the bypass (seasonal mean 112 ng/ml) and after entering the raceways (seasonal mean 160 ng/ml) were a consequence of passage through the bypass. Although smolts could delay in the reservoir beneath the separator for various periods of time before passing out into the short flume leading to the raceways, most fish sampled after entering the raceways had probably passed thorugh the bypass within the preceding few minutes to several hours.

Chinook salmon smolts undoubtedly attempted to swim against the water flow in the collection channel within the dam and in the bypass pipe leading from the dam to the holding facility. Vigorous swimming was indicated by elevated lactic acid concentrations in the blood of fish in post-bypass and initial raceway samples. The peak lactic acid concentration of 67 mg/lOO ml measured in fish present in the raceway for 45 minutes or less can be compared with peak lactic acid concentrations of 50-150 mg/lOO ml measured in the blood of rainbow trout after 15 minutes of continuous chasing in a shallow trough (Black et al. 1959, 1962). Fish arriving in the raceways were probably affected by fatigue as well as by the physiological effects of stress.

Cortisol levels dropped rapidly to 70 ng/ml (seasonal average) during the first 6-8 hours of raceway residence, indicating that holding of fish in raceways for short time periods was not highly stressful. Loading densities did not exceed 30 g/liter (0.25 pound/gallon) during our 1982 sampling.

Cortisol levels rose to 180-190 ng/ml in smolts loaded onto trucks or barges then declined to 99-117 ng/ml in three

of four groups of chinook salmon arriving at Bonneville by barge. Mean cortisol was much higher (214 ng/ml) in the fourth and last (April 30) barged group. The high cortisol level in this group was consistent with elevated cortisol levels observed in chinook salmon at other sampling points (gatewells, pre-separators, raceways) in late April. The cause of elevated stress indices in late April is not known, but this period coincided both with the peak of the chinook salmon run and with increased numbers of steelhead trout in the collection facility. Significantly, the descaling rate increased from 6% to 8% in the 2nd and 3rd weeks of April to 18% to 25% in the 4th week. We concluded that, under the conditions prevailing prior to late April 1982, barge transportation was a relatively unstressful step in the process of collection and transportation, although the loading procedure did elevate cortisol levels. Factors that may have contributed to elevated cortisol levels in barged fish in late April are unclear.

Mean cortisol was elevated to 249 ng/ml in the one sample of chinook salmon transported to Bonneville by truck. Sampling of chinook salmon trucked to Bonneville the following year (Congleton et al. 1984) confirmed that plasma cortisol concentrations typically exceeded 200 ng/ml in chinook salmon trucked to Bonneville.

The first rise in blood glucose seen in fish passing through the Lower Granite facility was in fish that had been held in raceways for 10-12 hours. This rise was a metabolic response to the earlier cortisol peak (and to the assumed concurrent adrenaline peak) occurring immediately after fish entered the raceways. Blood glucose was still elevated in two of the four barged groups (143, 152 mg/lOO ml) and in the one trucked group (176 mg/lOO ml) sampled after arrival at Bonneville, indicating that metabolic homeostasis had not been reestablished. However, in the remaining two groups of

barged fish, glucose had declined to levels similar to those observed in gatewell samples (118 mg/lOO ml in barged fish arriving on April 24 and 26).

Mean plasma Na+ concentrations declined from 150-180 meg/liter in fish sampled from gatewells at Lower Granite Dam to 120-130 meg/liter in fish sampled after transportation to Bonneville. Plasma Na+ (and other unmeasured ions) were lost as a result of stress-caused diuresis. Significantly, plasma Na+ did not return toward baseline (gatewell) levels during barge transport to the estuary: apparently the transit time of approximately 40 hours was not sufficient for physiological mechanisms to reestablish hydromineral equilibrium. Both trucked and barged fish were released into the estuary in a condition of ionic imbalance.

When fish were transported to Bonneville and held in fresh water, their plasma cortisol levels dropped to baseline levels after 48 hours. Elevated plasma glucose and depressed plasma Na+ levels, indicating secondary metabolic and osmoregulatory responses to stress, returned toward baseline levels more slowly. Possibly plasma glucose and electrolyte levels would not have completely recovered even after a week or more of freshwater recovery, since cortisol began to rise again after the second day of confinement. Less than 24 hours of recovery was needed for full restoration of ionoregulatory ability in sea water.

Holding of transported fish for 24 to 48 hours before release could improve post-release survival if elevation of stress indices is correlated with increased mortality in the lower river and estuary immediately after release. On the other hand, holding of transported fish would not be beneficial if the stress response contributes to mortality at a later time. For example, reduction of stress indices

before release would have little effect if the primary effect of the stress response is a lowering of resistance to endemic pathogens carried by many individuals in the population. In this event, mortalities might occur weeks or even months later.

The distinct die1 cycles in plasma cortisol and glucose observed in fish sampled below the bypass on April 14-15 are believed to have been related to the average duration of residence in the gatewells. Cortisol and glucose concentrations were lowest in fish sampled at 1900 and 2300 hours, roughly the period of peak movement into the facility. Because few holdovers from Pish entering the facility the previous night would still have been present, most fish must have entered the facrlity sortly before sampling. The low cortisol concentration in fish sampled at 0700 hours similarly may have corresponded to a secondary peak movement into the facility about dawn, although a drop in glucose levels was not seen in these fish. Glucose and cortisol concentrations were highest in fish sampled at midday (1100 and 1500 hours), when most fish leaving the gatewells were stragglers from the previous night rather than fish that had recently entered from the forebay.

The procedures used for microwire tagging at Lower Granite Dam elicited a strong stress response. No samples were taken to determine cortisol levels in the fish before they entered the marking building, but values ranging from 41 to 136 ng/ml were measured for fisn held in raceways under similar conditions at other times. Plasma cortisol was relatively high (168 ng/ml) in fish sampled immediately after anesthetization and sham marking, but rose further (to 284 ng/ml) after the first hour of recovery. The first value reflected a stress response to crowding of fish from an outside raceway into the marking building and holding there in a small tank (about 1.2 x 1.8 x 0.8-m deep) for up

to several hours. The value measured after one hour of recovery reflected additional stress imposed by anesthetization. The composite effect resulted in a more extreme stress response than that experienced by unmarked fish exposed only to routine collection and transportation procedure.

Summary

1. Chinook salmon were not highly stressed by diversion into gatewells on three of the four sampling dates in 1982: plasma cortisol concentrations in fish from gatewells were similar (40-60 ng/ml) to baseline cortisol concentrations in hatchery-reared chinook salmon. Plasma cortisol concentrations were higher (135, 154 ng/ml) in fish from gatewells on the fourth sampling date (April 30).

2. A die1 cycle in plasma cortisol and glucose concentrations in spring chinook salmon sampled after exiting the bypass pipe was attributed to delay in the gatewells or bypass system. Cortisol concentrations were lowest and glucose concentrations were highest (minimum stress response) during the peak of the evening migration: cortisol concentrations were highest and glucose levels were lowest (maximum stress response) during midday.

3. Passage from gatewells to raceways resulted in a large change in stress indices relativ to most other steps in collection and transportation. Only truck and barge loading elicited an equally large cortisol response.

4. Fish swam vigorously during bypass passage, as indicated by an elevated plasma lactate concentration

(67 mg/lOO ml, compared with 24 mg/lOO ml after overnight recovery in a raceway).

5. Plasma cortisol concentrations dropped rapidly during the first 6-8 hours in a raceway, then rose again after smolts were loaded onto trucks or barges.

6. Plasma cortisol concentrations declined during barge transportation on three of four dates but rose on the last (April 30) sampling date. Factors contributing to higher cortisol concentrations in fish passing through the collection facility in late April are unknown.

7. Plasma glucose concentrations rose and Na^+ concentrations fell during collection and transportation.

8. Procedures used for microwire tagging of smolts elicited a strong stress response.

9. Plasma cortisol concentrations returned to the baseline range within 24 to 48 hours after fish were transported to Bonneville. Plasma glucose and Na^+ concentrations recovered more slowly, but fish could tolerate full strength sea water in 24 hours or less.

4. CORRELATION BETWEEN STRESS INDICES AND PREDATOR AVOIDANCE ABILITY

Understanding the effects of the collection and transportation system on critical aspects of smolt performance is necessary for evaluation of the efficacy of pcssible improvements in the system. BY establishing a correlation between physiological stress indices (such as elevated plasma cortisol or depressed electrolytes) and impaired performance in the laboratory, and by then monitoring stress indices in transported smolts, an indirect means of assessing the potential performance of transported fish could be developed,

Ability to avoid fish predators was selected as a test of smolt performance because predation is known to be a major factor affecting survival of juvenile salmonids (Ricker 1941; Thompson and Tufts 1957;. Also, predatory fishes are abundant in the Columbia River between dams and near sites where transported fish are released (Thompson 1959; Uremovich et al. 1982).

Bioassays have been developed to evaluate the predator-avoidance performance of several species of juvenile salmonids exposed to fish predators. Barns (1967) observed the effects of several rearing regimes on the ability of juvenile sockeye salmon to avoid predation by cutthroat trout. Hatfield and Anderson (1972) tested the vulnerability of Atlantic salmon parr to large brook trout predators after exposure to several concentrations of two organic insecticides. Coutant (1973) tested the vulnerability of juvenile chinook salmon and rainbow trout subjected to thermai shock to predation by rainbow trout, and Snyder (1980) investigated the effects of starvation on

the predator avoidance ability of pre-smolt coho salmon exposed to cutthroat trout.

Poor physical condition has been linked to increased prey vulnerability under laboratory conditions (Herting and Witt 1967; Mauck and Coble 1971), but the relation between physiological stress indices and prey performance is poorly understood.

We attempted to determine if a relation exists between stress response indices and vulnerability to predation in juvenile chinook salmon. Crowding was used as a stressor because it is reproducible and is accompanied by little risk of physical injury. We hypothesized the following results:

1. Vulnerability of crowded fish to predation would increase as crowding density or duration increased.

2. Plasma cortisol concentrations would be positively correlated, and plasma Na^+ levels negatively correlated, with crowding density and duration.

3. Predation mortality would be positively correlated with plasma cortisol concentration above some "Threshold" concentration.

4. Predation mortality would be inversly related to plasma Na^+ concentrations below some "threshold" concentration.

Materials and Methods

Predator-prey bioassays were performed with five groups of chinook salmon prey, three predatory fish species, and two artificial environments (Table 4.1). All trials were

Table 4.1. Predator-prey combinations and test environments used for predator-prey bioassays September 1982 to October [illegible]983)

Stock of salmon	Prey National fish hatchery of origin	Age (year)	Total length range (mm)	Predator	Test environment	Test period
Klickitat fall chinook	Bage[illegible]m[illegible]	0	[illegible]0-[illegible]39	Smallmouth bass	Pool	9/30-10/28/82
Kooskia spring chinook	Kooskia	0	100-129	Northern squawfish Rainbow trout	Pools Channel	9/11-10/20/8[illegible] [illegible]/10-8/14/83
Kooskia spring chinook	Kooskia	0	110-139	Smallmouth bass	Pools	12/0[illegible]-12/14/82
Little White Salmon spring chinook	Dworshak	1	[illegible]20-[illegible]4[illegible]	Smallmouth bass	Pools	1/26-5/23/83
Rapid River spring chinook	Dworshak	0	[illegible]0-[illegible]3[illegible]	Rainbow trout	Channel	9/11-10/09/83

conducted at the Hayden Creek Research Station near Lemhi, Idaho.

Five circular swimming pools (2.4 m in diameter and 0.4 m deep) were utilized for trials with smallmouth bass and northern sguawfish predators. Cover for prey was provided by a sloping shelf of cobble, 5-15 cm in diameter (minimum water depth 15 cm), partially covered by a small shade tent. Cover for predators was provided by placing a second shade tent opposite the prey refuge (Fig. 4.1). Spring water was provided at 16 C and 11 C for bass and sguawfish, respectively, through the outlet from the pre-holding boxes. Four to six smallmouth bass (0.2 to 1.2 kg) or 8-9 northern sguawfish (0.3 to 2.0 kg) inhabited each predation pool. Predator-prey interactions were observed from a blind.

All trials with rainbow trout predators were carried out in an artificial stream channel (1.8-m wide x 1.2-m deep x 18.3-m long; Fig. 4.2). Cover for chinook salmon prey was provided by 2 riffles (1.8-m wide x 0.15-m deep x 1.2-m long) in the artificial stream. Boulders on the riffles allowed chinook salmon to move freely among the 3 pools but restricted the rainbow trout to a single pool.

During the tests, 28-30 large trout predators (0.3 to 1.3 kg each) were present in the channel. The channel was provided with 11 C spring water at 0.014 m3 per second. Chinook salmon that moved downstream could enter a trap at the lower end of the channel.

Smallmouth bass were obtained from Brownlee Reservoir on the Snake River. Northern sguawfish came from the St. Maries and Snake Rivers. Koocanusa strain rainbow trout were brood stock from the Hayden Creek Research Station. Both smallmouth bass and northern squawfish are potential predators on outmigrating chinook salmon smolts in the lower

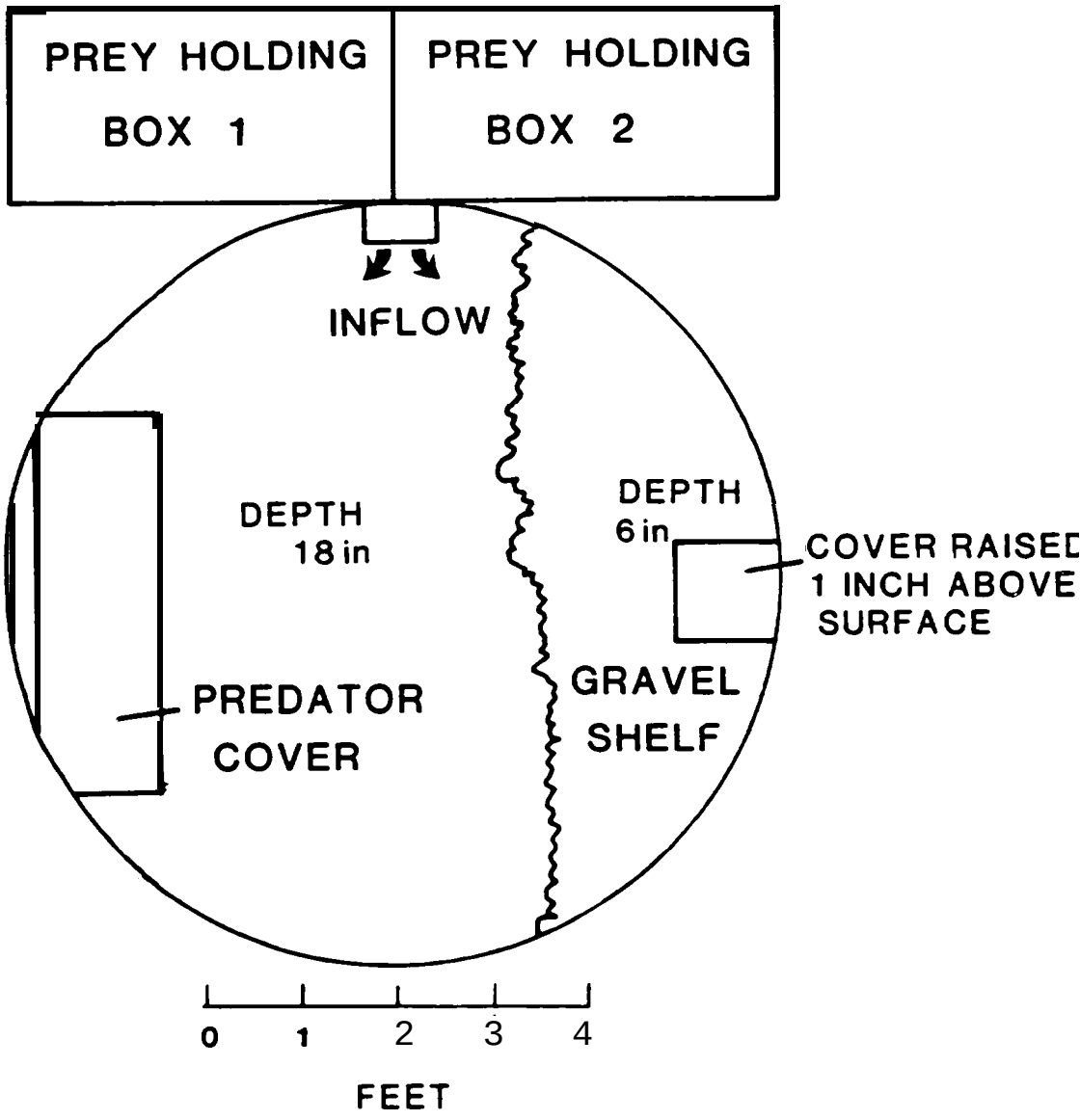


Figure 4.1. Overhead view of pool used in predator avoidance tests.

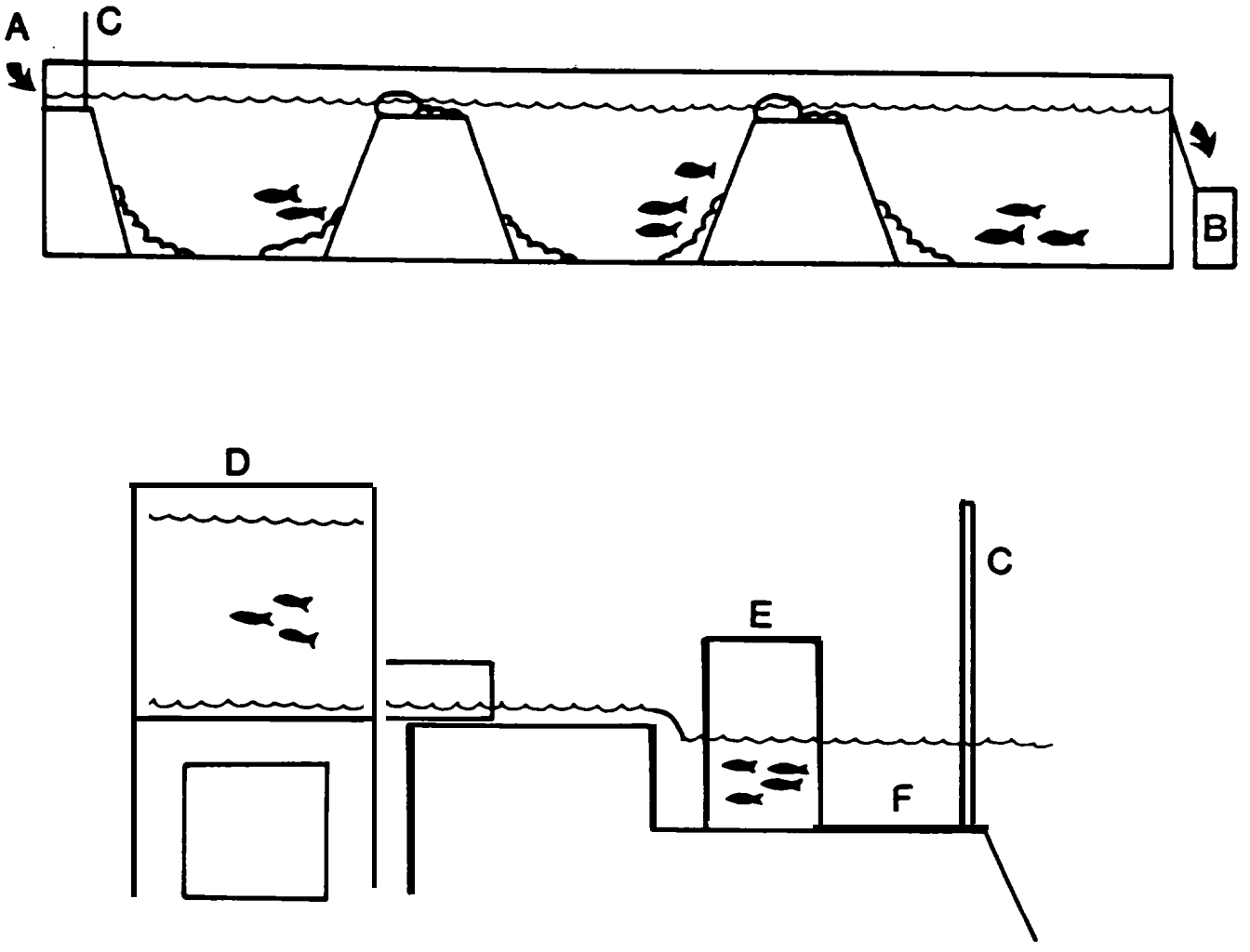


Figure 4.2. (Above) Artificial stream channel used for trials with rainbow trout as predators: A) incoming water supply, B) outflow spillway and migration trap, C) barrier screen preventing trout from escaping head of channel. (Below) Detail of head of channel: D) holding box for prey control group, E) crowding cage for prey treatment group, F) staging area for mixing of control and treatment group before release into the channel.

Columbia River (Uremovich et al. 1980). Koocanusa strain rainbow trout are native to Lake Koocasnusa in northern Montana: fish are the major component in the diet of large individuals. Predation trials were staged at 4-day intervals in an attempt to standardize predator hunger and behavior.

Upon arrival at the station, all chinook salmon were graded into 10-mm length classes and cold branded (with liquid nitrogen) on the right or left side between the dorsal fin and the lateral line. Each group of fish was then placed in a separate holding tank for a minimum of 96 hours to recover from handling and branding. Juvenile chinook salmon were fed at levels recommended by standard feeding chart.

To begin a run (l-3 simultaneous trials), one group of right- or left-branded prey was randomly selected as the treatment (crowded) group. A total of 30-70 fish were netted from the holding tank, weighed, and loaded into an adjustable volume cage submerged in a trough with running water (smallmouth bass trials) or submerged at the head of the artificial stream channel (rainbow trout trials). Prey were also crowded for northern squawfish trials at the head of the stream channel to prevent thermal shocking of prey at the trial of introduction to pools (bass and squawfish were held at different temperatures). Control fish (uncrowded and unstressed) were transferred to prey holding tanks (Fig. 4.1, 4.2) 96 hours befcre the start of a trial. After fish had been crowded for a designated period (Table 4.2), 10 fish from both test and control groups were removed and anesthetized in 50 mg/liter MS-222. Blood samples were obtained by severing the caudal peduncle of subsampled fish and collecting blood with heparinized capillary tubes. Plasma samples were immediately centrifuged and frozen for later analysis.

Table 4.2. Loading densities and durations for trials successfully completed with each stock of chinook salmon prey (September 1982 to October 1983).

Stock of salmon	Loading densities (kg/cubic meter)	Crowding durations (hours)	Number trials completed	Predators	Environment
Klickitat fall chinook	128	96	15	Smallmouth bass	Pools
Kooskia spring chinook	385	48, 96, 192, 240	10	Squawfish	Pools
			12	Rainbow trout	Channels
Kooekia spring chinook	128, 193	96	12	Smallmouth bass	Pools
Little White Salmon spring chinook	128, 193, 385	24, 48, 96	42	Smallmouth bass	Pool5
Rapid River spring chinook	385	48, 96, 240	5	Rainbow trout	Channels

For predation trials with bass and sguawfish, 10 fish were dip-netted from the crowding cage, transferred in a 5-gallon bucket to the appropriate prey holding tank, and allowed to mix with control fish for about 2 minutes. While test and control fish were mixing, a net on a rigid frame was placed in the pool to restrain the bass in one section of the pool. Sguawfish frightened easily and hid beneath cover in the presence of an investigator, so restraining nets were not necessary. The mixed group of test and control fish was released into the pool by opening a sliding door on the prey holding tank. Prey were allowed to acclimate to the pool at least 10 seconds before the restraining net was removed and the bass released. Predation was allowed to proceed for as long as 24 hours; all surviving prey were then netted from the pool and identified. Figure 4.3 summarizes these procedures.

For stream channel trials, 25 control and 25 test prey were released directly into a staging area at the head of the channel and allowed to mix (Fig. 4.2). After 2 minutes, a screen at the end of the staging area was raised and prey were released into the channel. As soon as all prey were in the channel, the screen was lowered to prevent them from returning to the staging area. Predator-prey interactions and differences in behavior between treatment and control fish were viewed through a series of windows along both sides of the channel. After 24 hours, the water level in the channel was dropped, and surviving prey were netted and identified.

The statistic used to express the difference in predation rates upon the two groups of fish (crowded and controls) was the ratio dp, previously used in the predation studies of Barns (1967) and Coutant (1973):

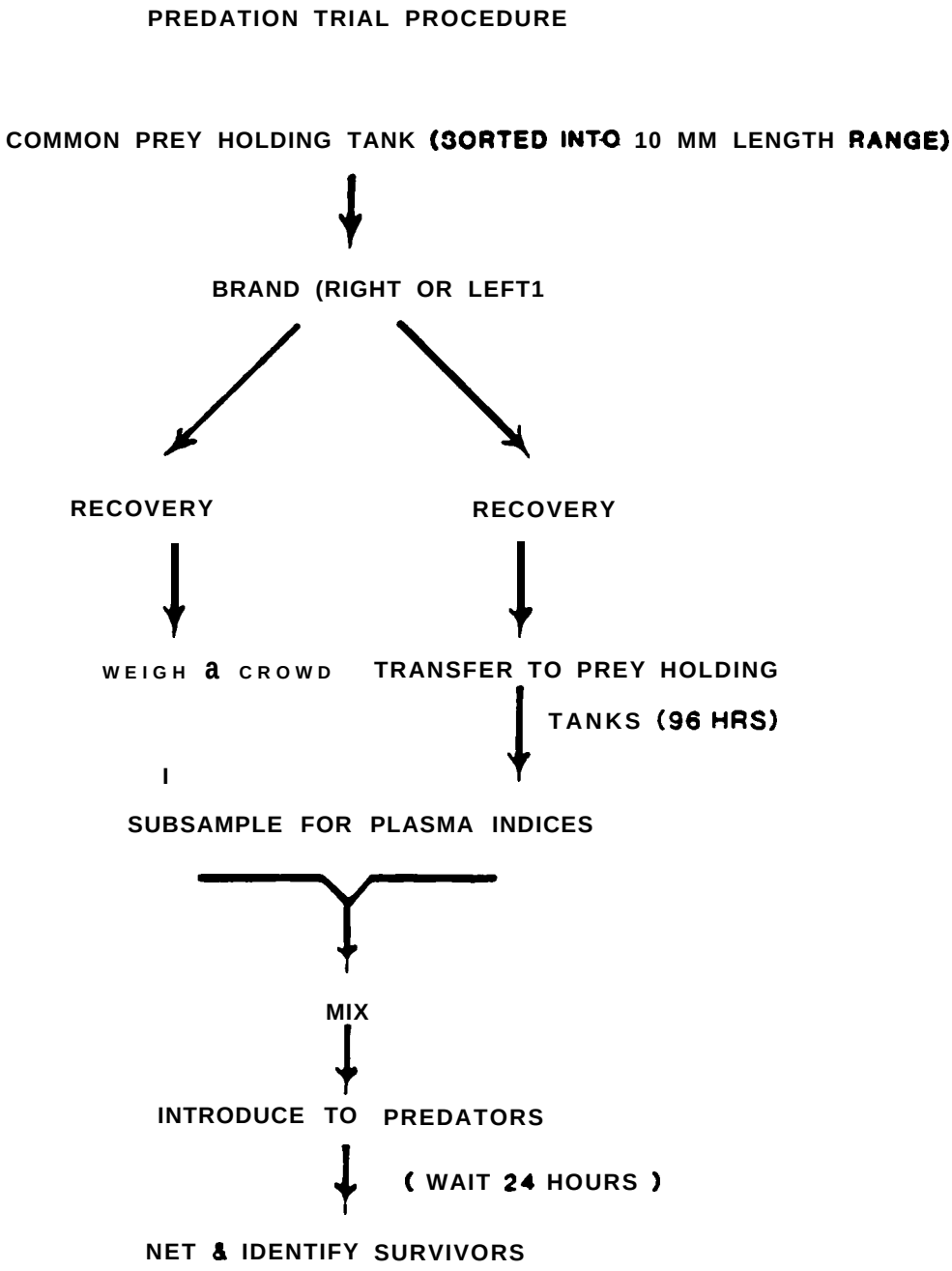


Figure 4.3. Predation trial procedure for introducing crowded and control (uncrowded) chinook salmon prey to fish predators.

$$dp = i_t / i_c$$

where i_t and i_c are the instantaneous mortality rates of the test and control groups. The instantaneous mortality rate for one unit of time is given by:

$$i = -\log_e S$$

where S = survival ratio, i.e. (n at finish)/(n at start).

Linear correlation **was** used to test for significant relationships between plasma cortisol or **Na+** concentrations, and dp. Curves were fitted to graphic data with the assistance of a SAS PROCREG program. Chi-square analysis was used to test for significant differences in survival between test and control groups.

Plasma cortisol samples were **analyzed** by a radioimmunoassay procedure developed at Oregon State University. Some samples were analyzed there and some were analyzed in our laboratory by the same procedure. Cortisol concentration determinations had within- and between-run coefficients of variation of 10%-11% (determined for **samples** analyzed at the University of Idaho). Plasma sodium **was** monitored in only three of the chinook salmon prey groups tested (Rapid River, Little White Salmon and Age 0 Kooskia stocks). Concentrations were measured with a flame photometer (Instrumentation Laboratory model 143); within- and between-run coefficients of variation were 5%.

Results

Smallmouth Bass **as** Predators in Circular Pools

Behavior of Predators and Prey: After introduction into circular pools containing smallmouth bass, juvenile

chinook salmon formed loose aggregations near the center of the pool along the deep water margin of the gravel shelf. An act of predation was usually initiated by the slow movement of a single smallmouth bass from beneath the plywood predator refuge to the center of the pool. Attacks were launched when a prey fish failed to maintain a critical distance between itself and the approaching bass. Strikes were rapid and highly efficient, providing the prey little opportunity for escape. Prey that avoided the bass when initially approached were rarely attacked. Surprisingly, prey that behaved in an overtly abnormal manner were also rarely attacked. Moribund fish were not used in tests, but in several trials prey lost their aguilibrium after introduction to the pool (due to the delayed effects of stress or to injury from a previous attack) and swam haphazardly throughout the pool for several hours, but were totally ignored by the predators. In 2 trials prey that died during the trial were untouched by predators.

Predation on Sorins Chinook Salmon, Little White Salmon Stock: Forty-two trials were completed with Little White Salmon spring chinook salmon after the fish had been crowded at densities of 128, 193, and 385 g fish/liter water (8, 12, and 24 pounds/cubic foot) for durations of 24, 48, or 96 hours. Mean cortisol concentrations ranged from 8 to 226 ng/ml for crowded fish and from 8 to 187 ng/ml for controls. Cortisol concentrations were highly variable at the two higher loading densities for all crowding durations, but were generally greater than levels observed in fish crowded at 128 g/liter (Fig. 4.4). Absolute and relative (absolute minus control) mean cortisol concentrations for each set of three simultaneously run trials were tested for linear correlation with depredation ratios (Fig. 4.5). In one run, blood samples were not taken from control fish because of a mechanical failure: consequently, only absolute cortisol concentration could be analyzed for the corresponding

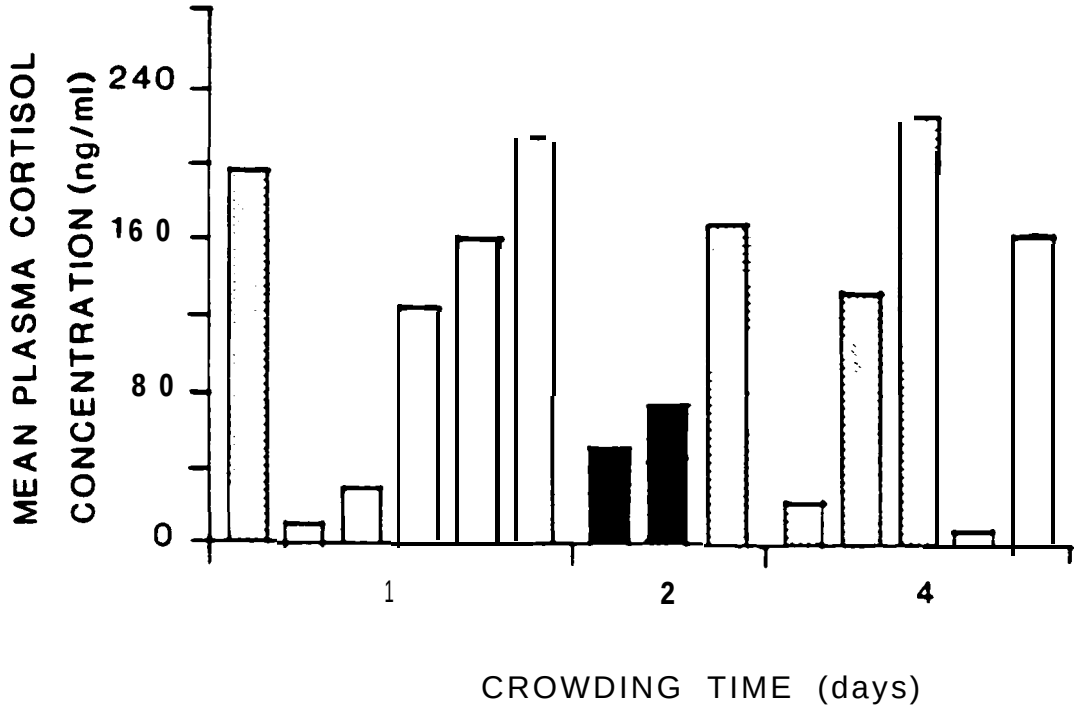


Figure 4.4. Crowding densi:ies,durations, and mean plasma cortisol concentrations observed in Little White Salmon chinook prey. Each bar represents one subsample of ten fish (one run). Slack bars = crowding density of 128 g/liter; shaded bars = 193 g/liter; open bars = 385 g/liter.

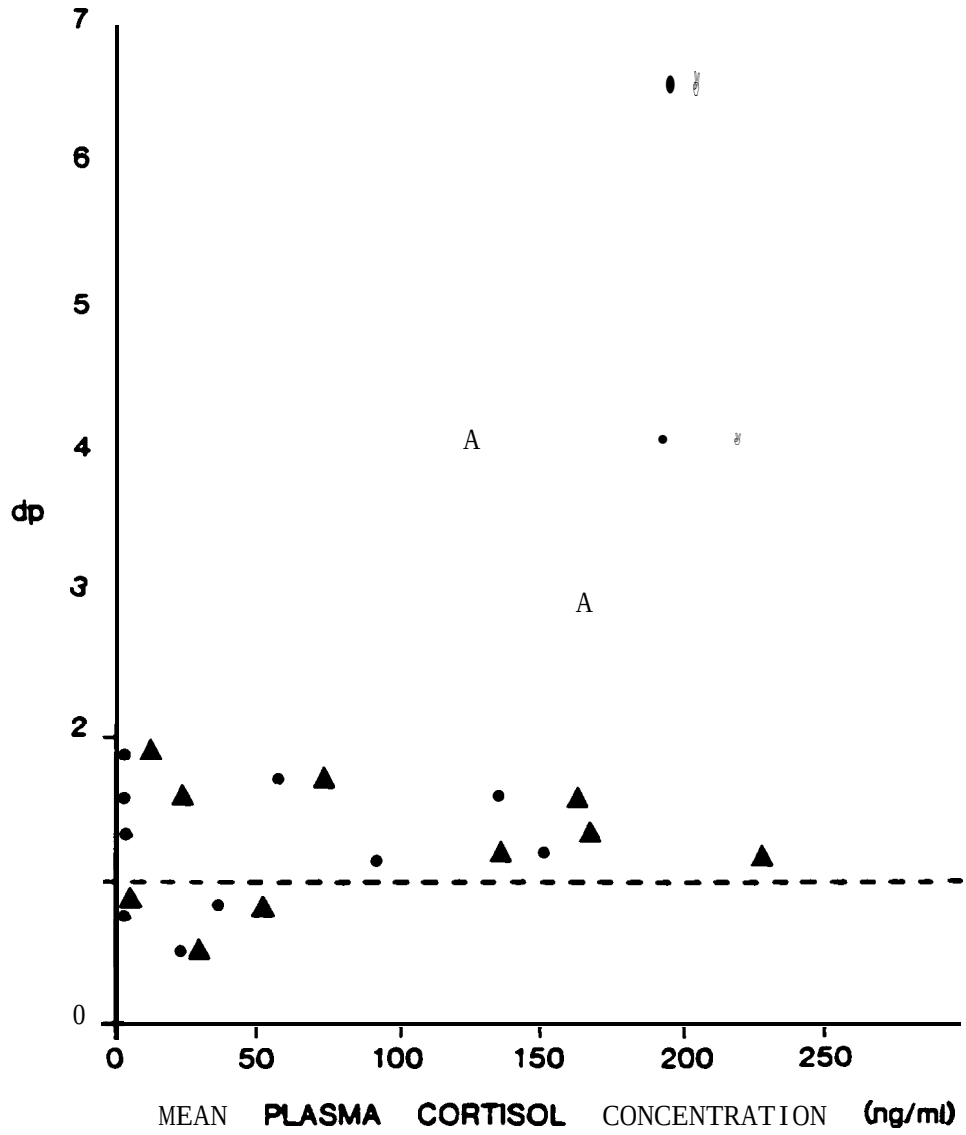


Figure 4.5. Mean plasma cortisol concentrations and depredation ratios (dp) for Little White Salmon spring chincok (age 1) and smallmouth Sass predators. Triangles indicate mean cortisol concentrations in treatment groups (mean of three Daired trials); circles indicate relative (mean treatment-mean control) cortisol concentrations.

treatment group. Linear correlations were significant ($0.01 < P < 0.025$, $r = 0.51$) for both mean and relative mean cortisol concentrations of treatment fish. When all trials with mean cortisol concentrations exceeding 125 ng/ml were treated as one group, differential predation was highly significant ($P < 0.001$, chi-square analysis). Differential predation was not evident when all treatment groups with mean cortisol concentrations below 125 ng/ml were analyzed as a group. Prey crowded at 128 g/liter (duration held at 48 hours) showed no reduction in ability to avoid bass predators (overall dp = 1.1, 6 trials) and had relatively low cortisol concentrations (means of 51 and 73 ng/ml for 2 runs). Prey loaded at 193 g/liter (3 trials) for 24 hours had subsampled mean cortisol titers of 198 ng/ml and were captured by predators at greater rates (dp = 6.6) than at greater crowding durations (48-hour dp = 1.4, 3 trials: 96-hour dp = 1.3, 9 trials). Chinook salmon crowded at 385 g/liter were more vulnerable than controls after crowding for 24 hours (dp = 1.8, 15 trials) and 96 hours (dp = 1.6, 6 trials).

Mean plasma Na concentrations (meg/liter) ranged from 78-148 for crowded fish and from 139-155 for controls. An inverse correlation between plasma Na^+ concentrations and prey vulnerability was not significant ($0.25 > P > 0.10$, $r = 0.31$) but in seven of eight runs (21 trials) in which mean plasma Na^+ concentrations fell below the range of means observed for controls, treatment prey were more vulnerable than controls **(Fig. 4.6).**

Predation on Spring Chinook Salmon, Kooskia Stock: Twelve trials were completed with Kooskia spring chinook salmon prey crowded at 128 and 193 g/liter for 96 hours. Cortisol levels were not greatly elevated in any treatment group (range 22-95 ng/ml treatment, 14-82 ng/ml controls) but they appeared to be related to observed depredation

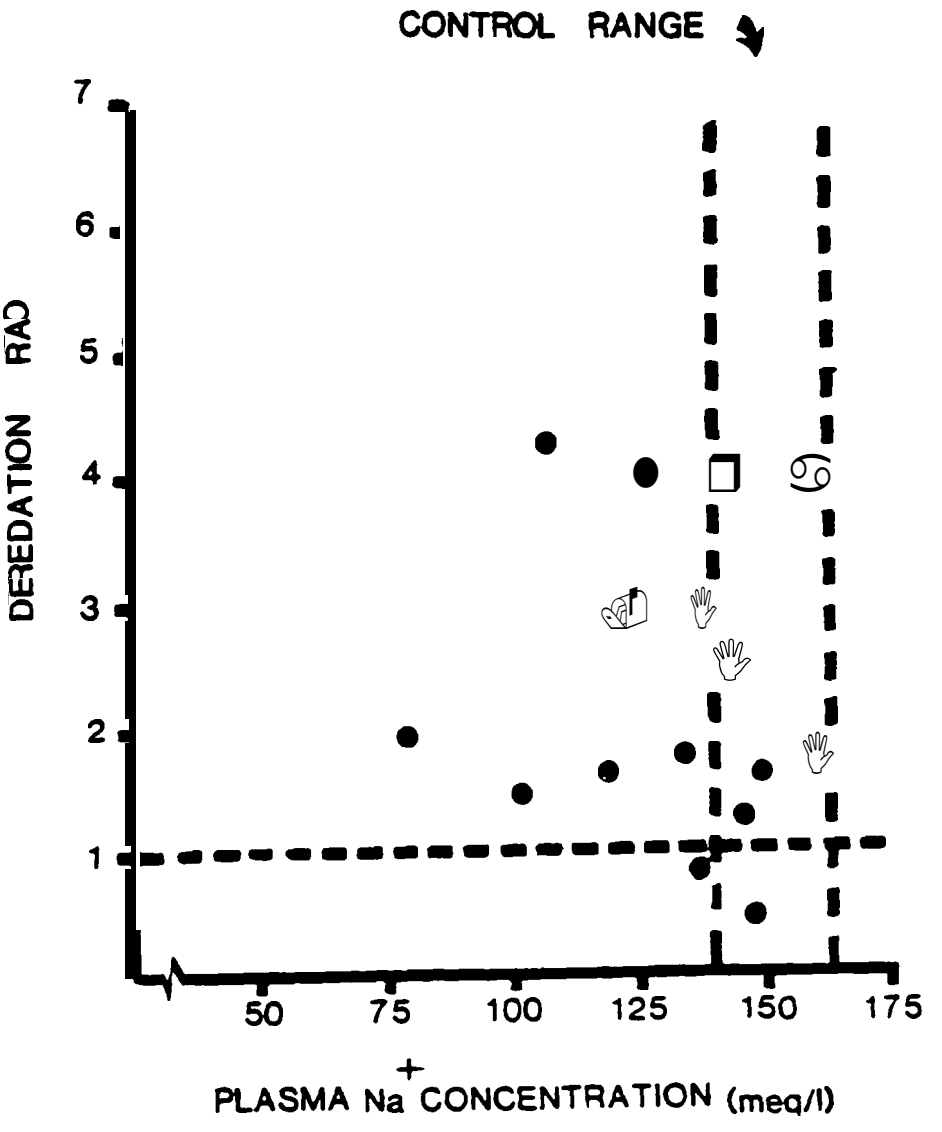


Figure 4.6. Mean plasma Na+ concentrations and depredation ratios (dp) observed in Little White Salmon chinook prey. Vertical lines indicate range of control means.

ratios ($r = 0.88$, $0.1 > P > 0.05$; Fig. 4.7). Although differential predation was not observed at 128 g/liter, it was significant at 193 g/liter ($0.01 < P < 0.05$, chi-square analysis, 1 df). Depredation ratios rose from 1.0 at 128 g/liter to 3.3 at 193 g/liter.

Predation on Fall Chinook Salmon, Klickitat Stock: All fall chinook salmon prey (15 trials) were crowded at 128 g/liter for 96 hours. Mean plasma cortisol concentrations ranged from 18 to 153 ng/ml for treatment groups and from 16 to 65 ng/ml for control groups. Significant differential predation was not observed with this group of chinook salmon prey ($0.75 < P < 0.90$). Depredation ratios remained near 1.0 over the range of observed cortisol concentrations in crowded groups; this was also true when differences between mean cortisol levels in treatment and corresponding control groups ("relativel' mean cortisol levels) were considered (Fig. 4.8).

Rainbow Trout as Predators in Stream Channels

Behavior of Predators and Prey: Interactions between chinook salmon and rainbow trout differed markedly from those between chinook salmon and smallmouth bass. Upon introduction to the head of the stream channel, chinook salmon formed a single large school or aggregation at the head of the uppermost pool. Trout would swim repeatedly through this aggregation searching for salmon that behaved differently than the rest of the school when approached. These prey often swam in a different direction than the rest of the school, darted excitedly when approached, or failed to respond to the predator. Once an attack was launched on an individual fish, a number of predators often participated in the pursuit. Attacks often ended unsuccessfully when the attacked prey reached the refuge of one of the two riffle areas or re-entered the school of chinook salmon.

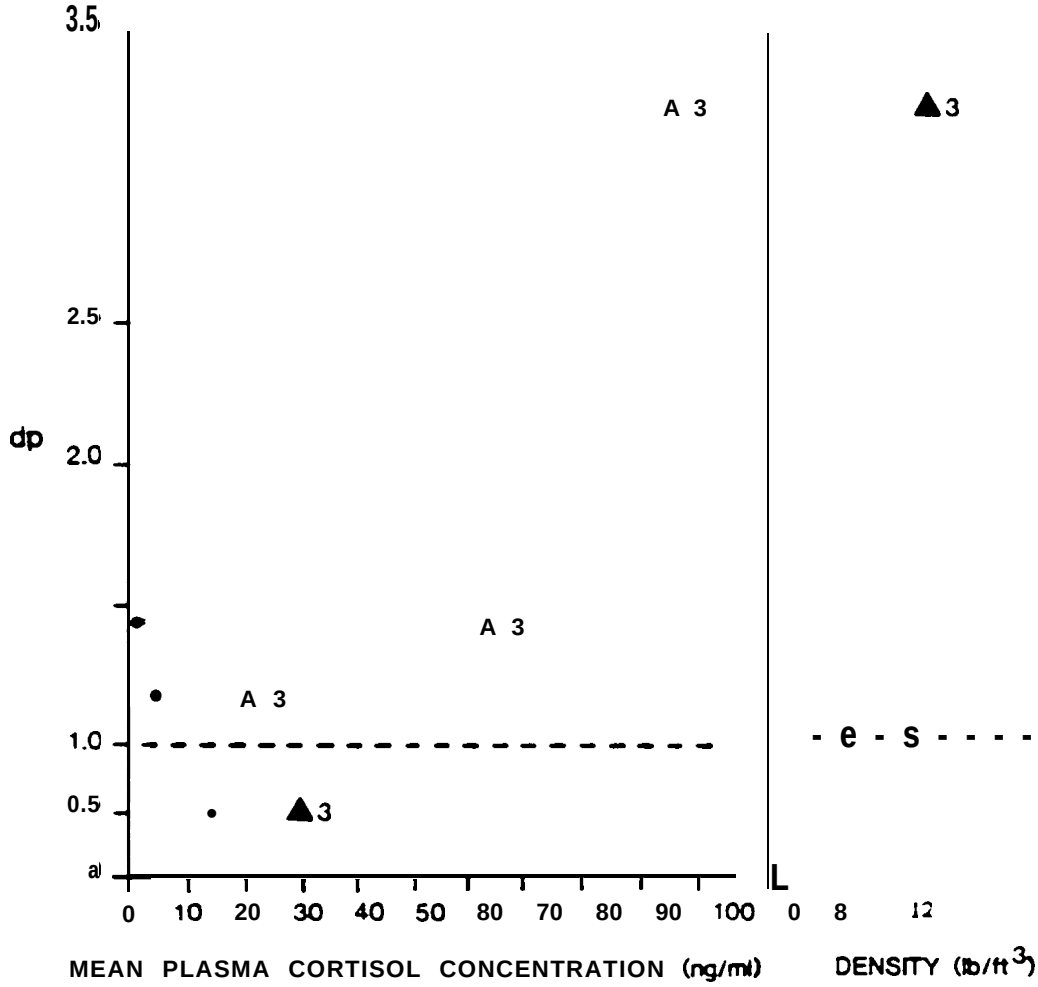


Figure 4.7. Mean plasma cortisol concentrations, loading densities and depredation ratios (dp) fcr Xooskia saring chinook and smallnouth bass predators. Test fish were crowded at 128 or 193 g/liter fcr 96 hours. Triangles indicate mean treatment cortisol concentrations in treatment groups; circles indicate relative (mean treatment-mean control) cortisol concentrations. Numerals indicate number of simultaneous trials.

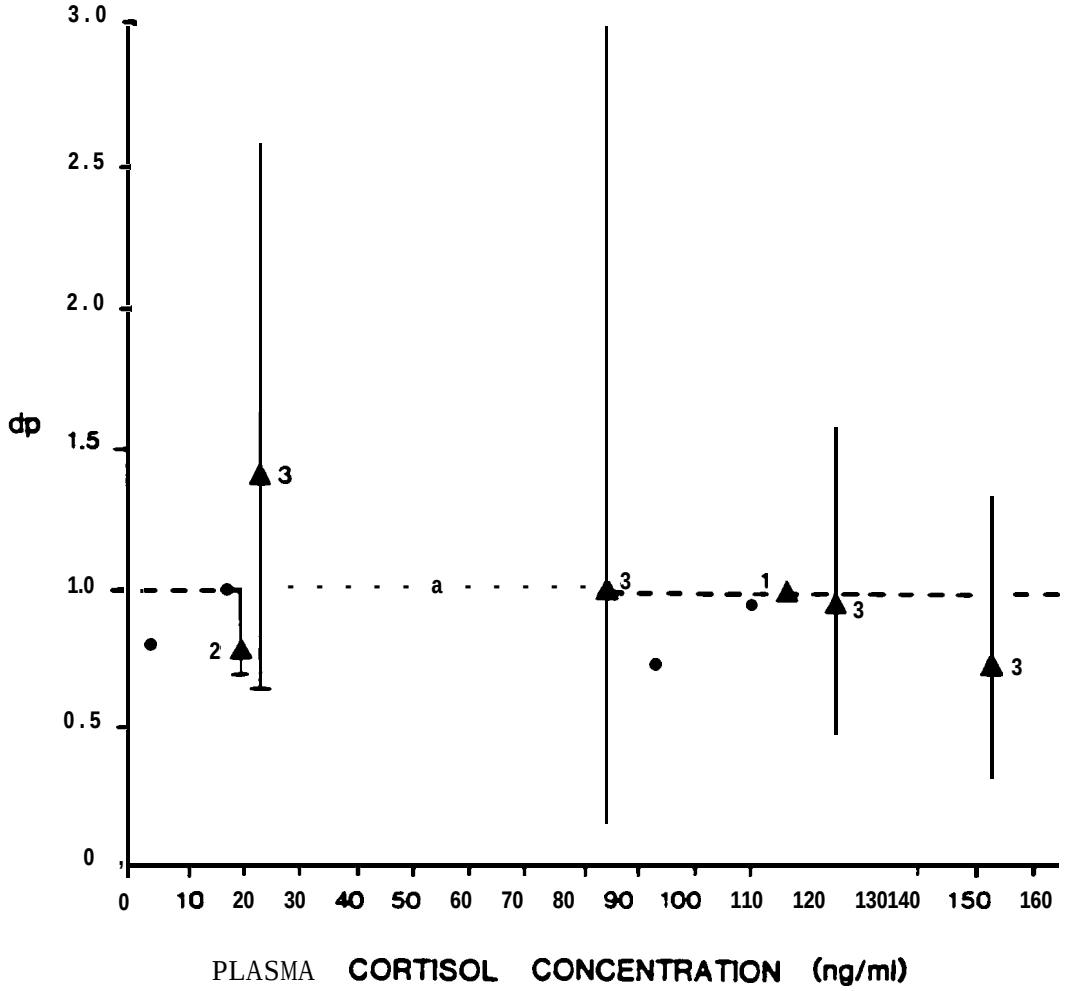


Figure 3.8. Mean plasma cortisol concentrations and depredation ratios (dp) for Klickitat fall chinook and rainbow trout predatcrs. Test fish were crowded at i28 g/liter for 96 hours. Tr'langles indicate mean cortisoi concentrations in treatment groups; circles indicate relative (mean treatment-mean control) cortisol values. Numerals indicate number cf simultaneous trials and vertical lines indicate dp range for simultaneous trials.

Predation on Spring Chinook Salmon, Kooskia Stock (Age 0): Twelve trials were completed with Kooskia age 0 chinook salmon prey. Treatment fish were held at 385 g/liter for 2, 4, 8, or 10 days. Mean cortisol concentrations for treatment groups ranged from 11 to 237 ng/ml and for control groups from 4 to 65 ng/ml. Mean plasma cortisol concentrations did not increase consistently as duration of crowding increased (Fig. 4.9). Absolute and relative mean cortisol concentrations were tested for correlation with the observed depredation ratios (Fig. 4.10). Data for 2 trials were not analyzed because of very low total prey consumption (<15% combined treatment and control fish eaten). Significant correlations existed between cortisol concentrations of treatment fish and dp ($0.01 < P < 0.025$, $r = 0.82$) and relative mean cortisol concentrations and dp ($0.025 < P < 0.05$, $r = 0.60$). When all trials in the upper half of the observed range of cortisol values were treated as a group (>125 ng/ml) differential predation was highly significant ($P < 0.001$). When all trials with prey exhibiting mean cortisol concentrations elss than 125 ng/ml were grouped similarly, crowded prey were still more vulnerable than controls, but differences were insignificant ($0.25 > P > 0.1$). Differential predation was also tested at each crowding duration by treating all trials at a given duration as a group. Significant differential predation occurred at both the 4-day crowding duration ($0.025 < P < 0.05$), due largely to 1 trial with very high dp, and 10-day crowding duration ($P < 0.001$, Fig. 4.11). In tests of 2 and 8 days, differential predation was not evident.

In only 4 of the 10 trials were the Na^+ concentrations in crowded fish below the range of means observed for controls. In each of these trials, however, crowded prey were more vulnerable to predation than were unstressed controls (Fig. 4.12).

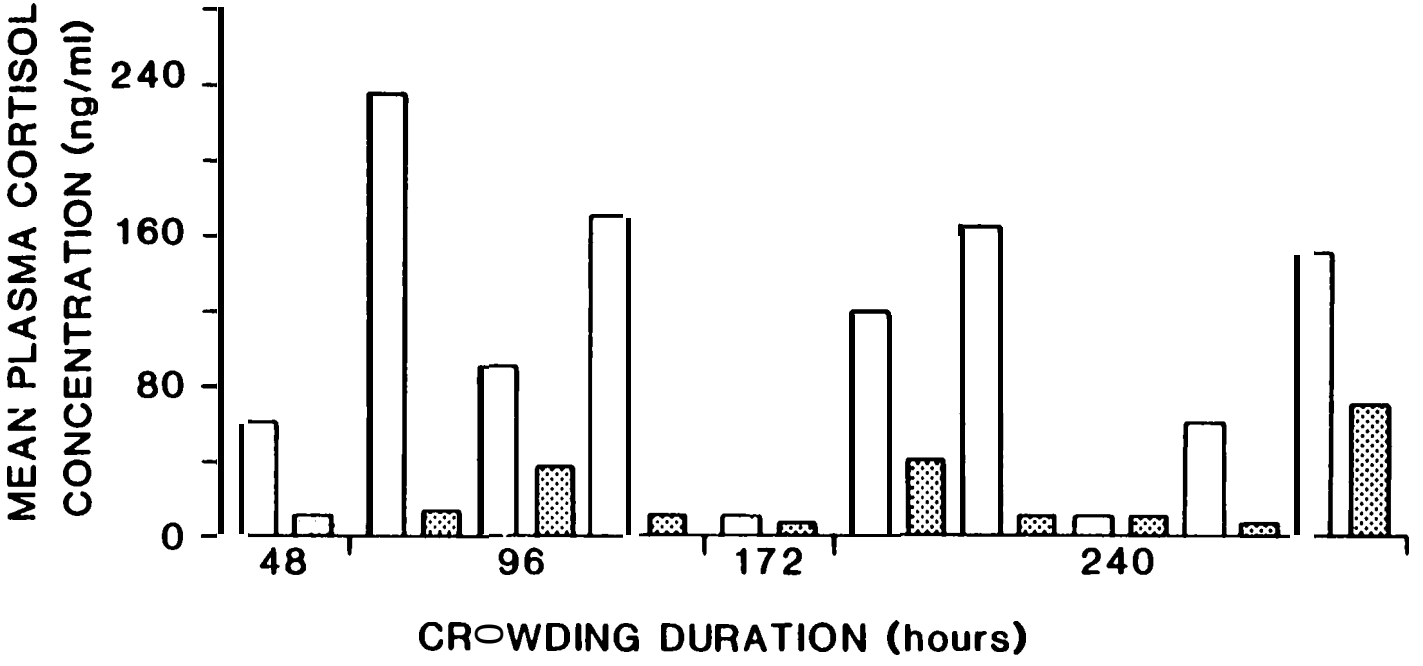


Figure 4.9. Relationship between crowding duration and mean plasma cortisol concentrations for Kooskia spring chinook at introduction into stream channels with rainbow trout predators. Open bars are means for crowded fish; shaded bars represent corresponding control means. Fish were crowded at 385 g/liter.

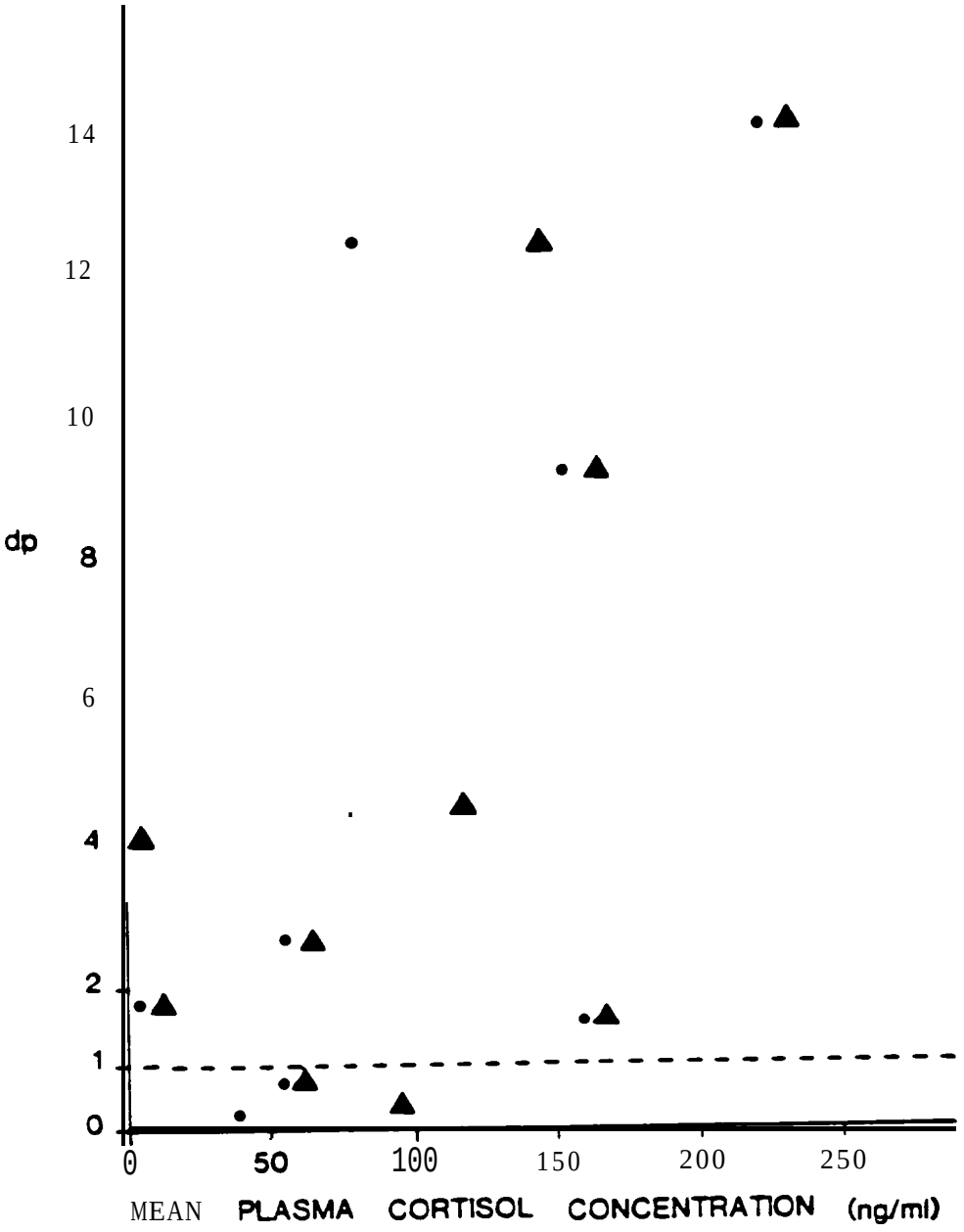


Figure 4.10. Mean plasma cortisol concentration ratios (dp) for Kooskia spring chinook and rainbow trout predators in stream channel triais. Triangles indicate mean cortisol levels in treatment groups (one trial each); circles indicate relative (mean treatment-mean control) cortisoi values.

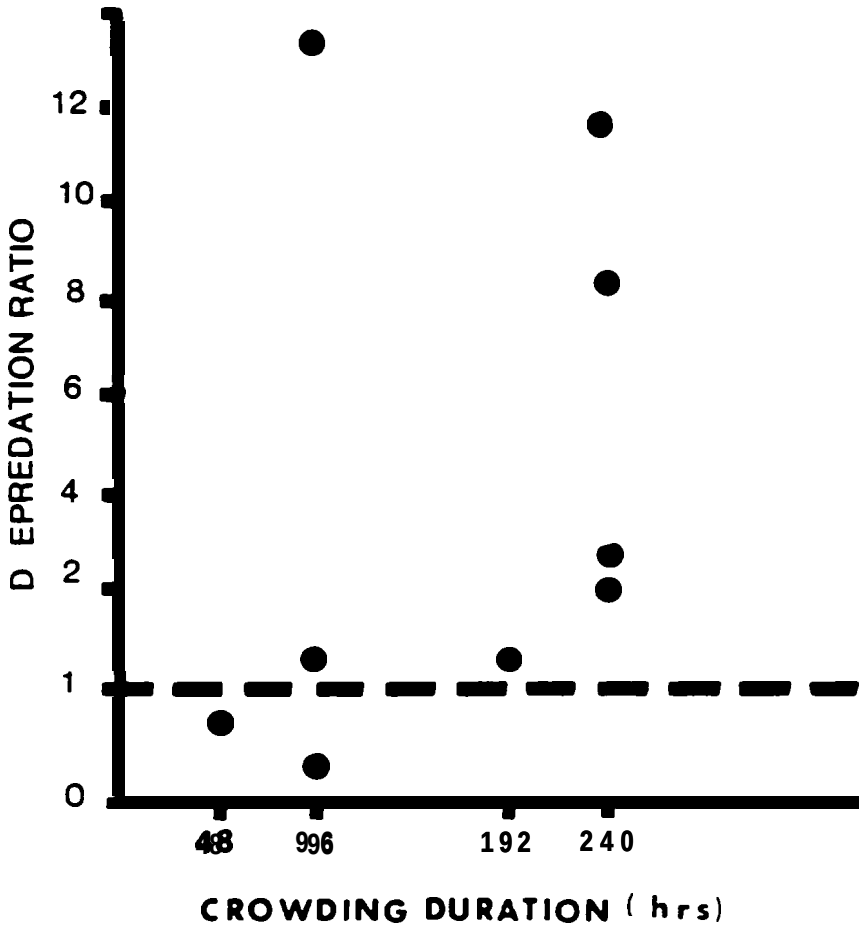


Figure 4.11. Relationship between crowding duration and depredation ratios (dp) for Kooskia spring chinook and rainbow trout predators in stream channel trials. All treatment fish were crowded at 385 g/liter.

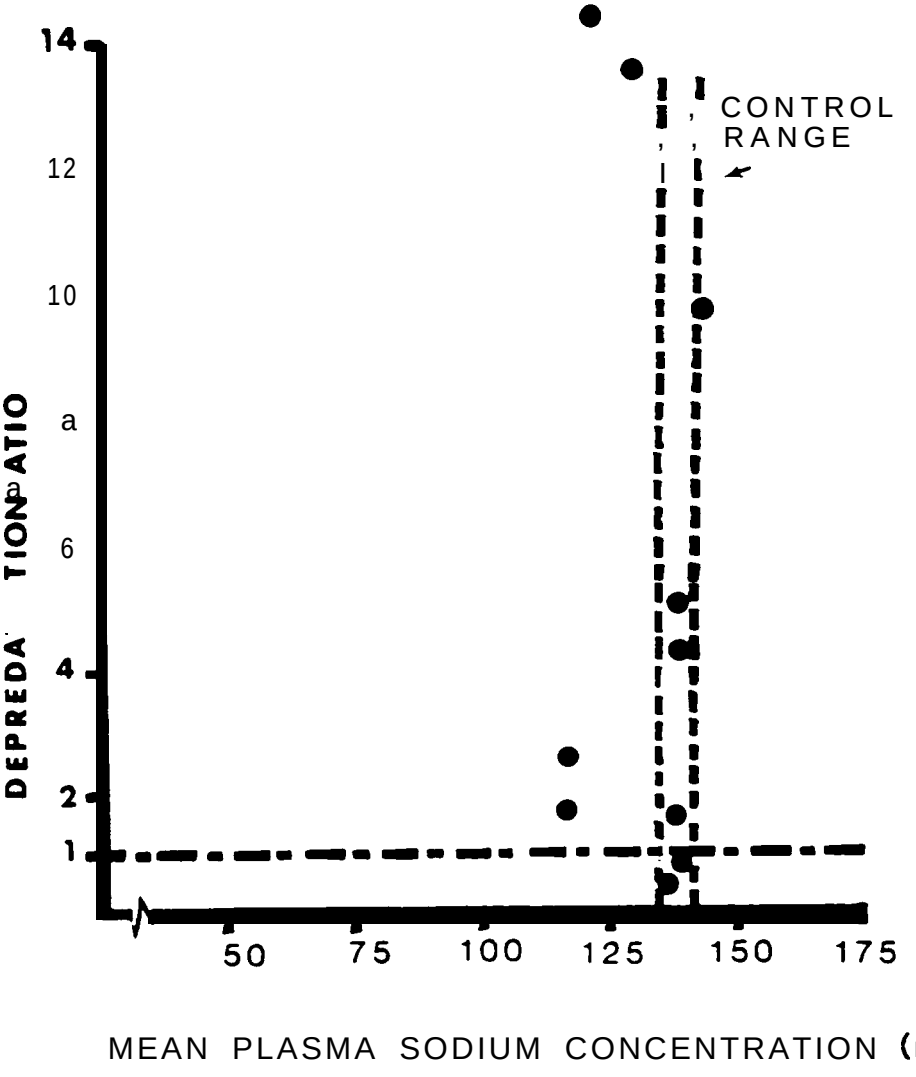


Figure 1.12. Plasma Na concentrations and depredation ratios(dp) observed in Kooskia chinook prey. Vertical lines indicate range of control means.

Allowing prey fish to volitionally outmigrate from the stream channel could have had an impact on resulting prey survival if crowded and control fish outmigrated at different rates, because prey fish entering the trap at the lower end of the channel were no longer exposed to predators. However, outmigration rates were similar for crowded and control groups at all durations tested (Fig. 4.13). The largest difference in observed rates (for fish crowded 96 hours) was insignificant ($0.1 < P < 0.9$, chi-square, 1 df).

Another factor affecting prey availability was location of prey within the stream channel. The two riffle sections in the channel provided refuge for prey fish due to the inability of predators to enter these shallow areas. More stressed than control prey were observed on these riffles over all durations tested, and prey fish used riffles more extensively as crowding durations were extended (Fig. 4.14). Additionally, stressed prey were occasionally observed at the water surface or in contact with the substrate, behavior infrequently observed in control prey (Fig. 4.15).

Predation on Spring Chinook Salmon, Rapid River Stock: Five trials were completed with Rapid River chinook salmon prey and rainbow trout predators in the stream channels. Crowding density was held at 385 g/liter for 2, 4, and 10 days. Plasma cortisol concentrations were not greatly elevated (treatment range 22-91 ng/ml, control range 8-47 ng/ml) were unaffected by crowding duration (Fig. 4.16), and were not highly correlated with dp ($0.25 < P$, $r = 0.39$, Fig. 4.17). When depredation ratios were tested for significance at each crowding duration, differential predation had occurred with 10 days crowding (chi-square test, $P < 0.001$) and was insignificant at 2 and 4 days (Fig. 4.18).

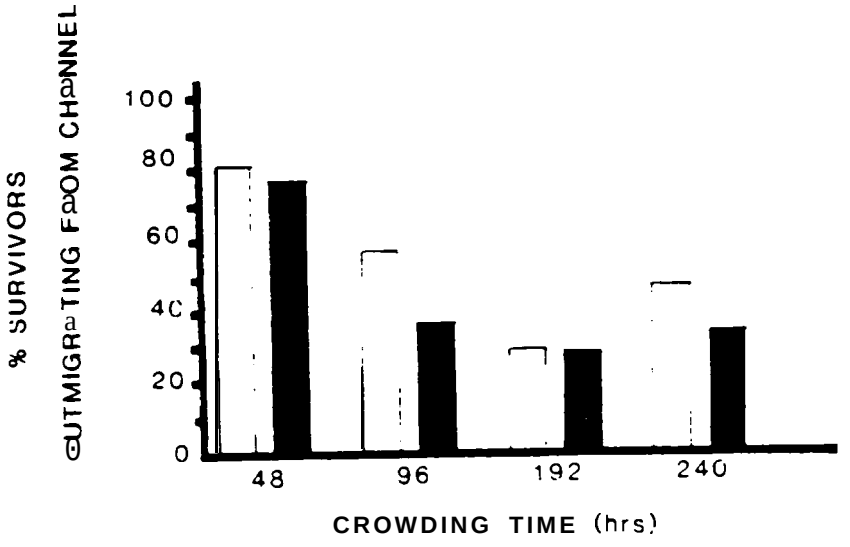


Figure 4.13. Relative outmigration of Koskia age 1 chinook prey from the experimental stream channels. Open bars indicate outmigration of crowded fish and shaded bars represent outmigration of controls.

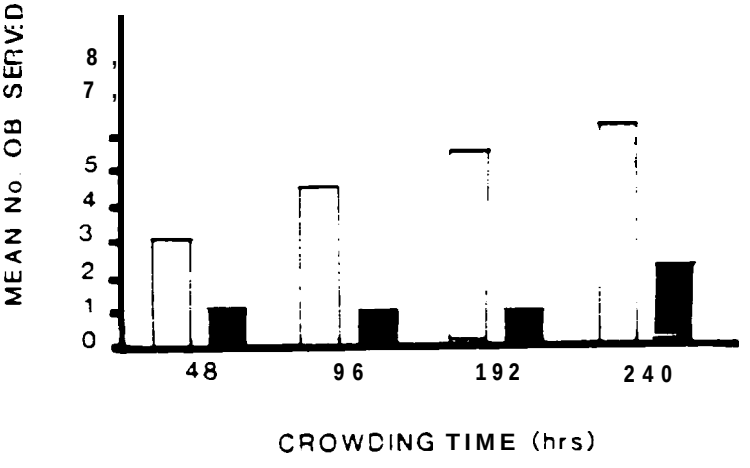


Figure 4.14. Mean number of Koskia age 1 prey observed in riffle sections of artificial stream channel during trials with rainbow trout predators. Open bars indicate crowded fish. Shaded bars indicate control fish.

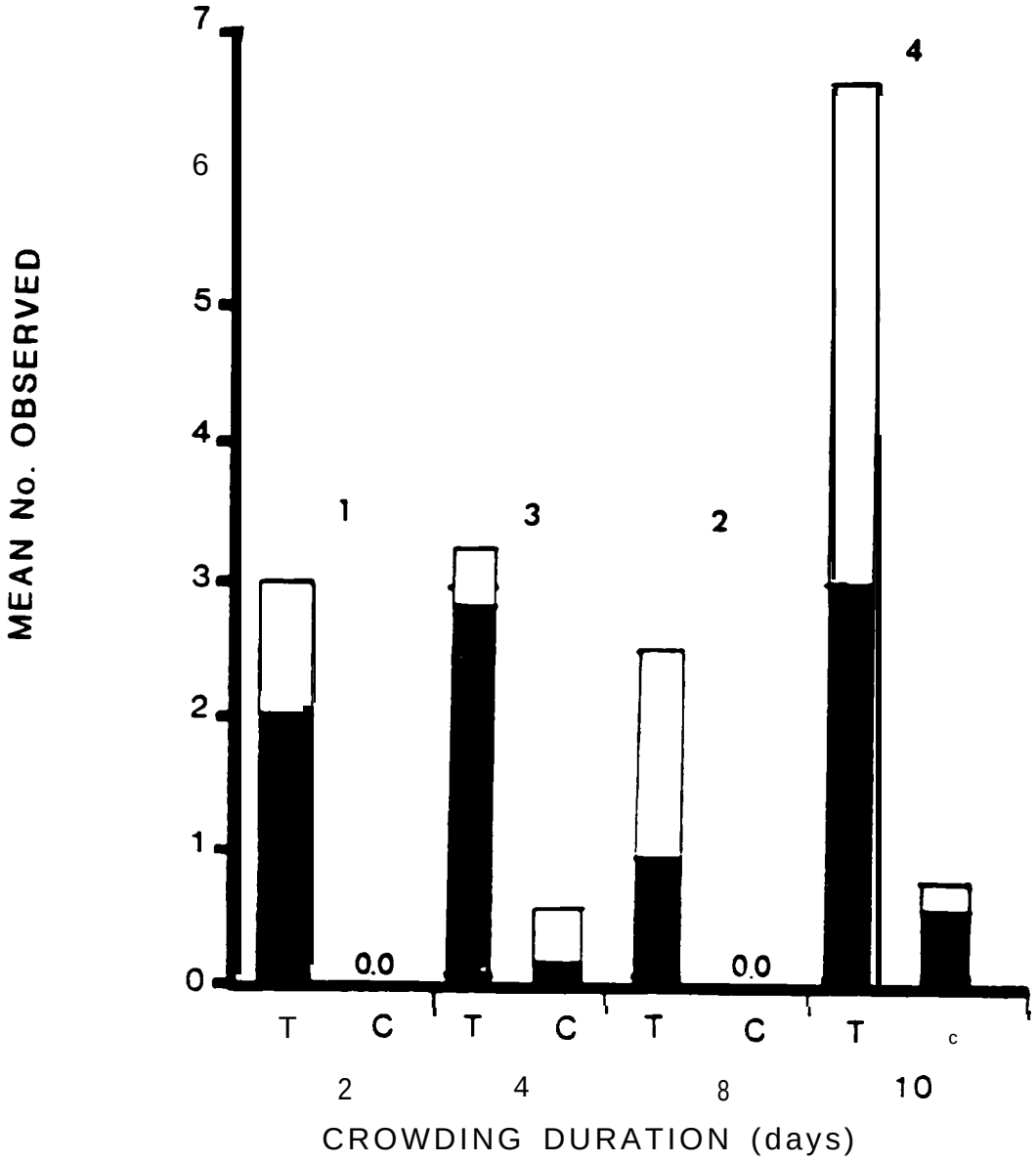


Figure 4.15. Mean number of crowded and control Kcoskia (age 0) prey observed at the water surface or in contact with the channel substrate. Open Cars indicate fish observed at the water surface; shaded bars indicate fish in contact with the substrate. T = treatment fish: C = control fish. Numbers at the top of *bars indicate number of trials at each crowding duration.

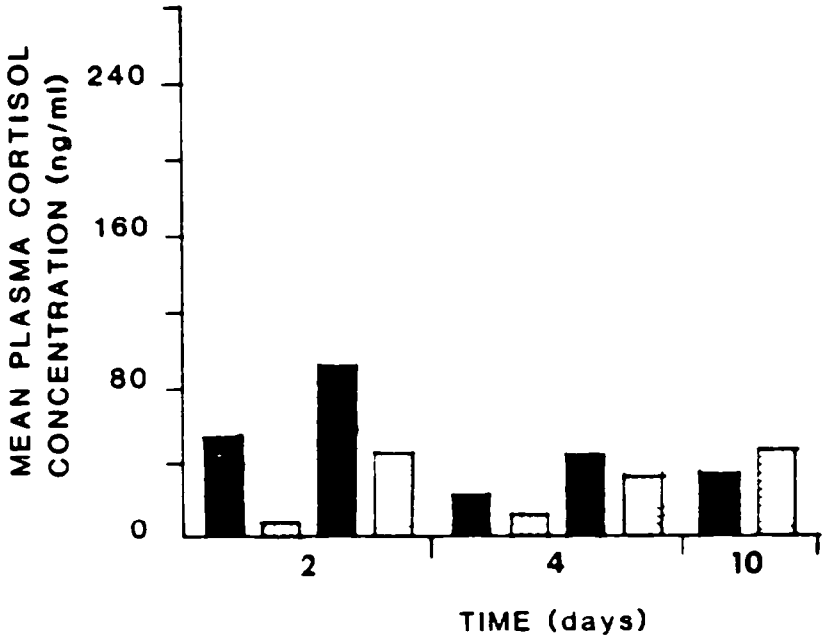


Figure 4.16. Mean cortisol concentrations observed at each crowding duration with Rapid River chinook prey. Shaded bars = crowded fish; light bars = corresponding controls.

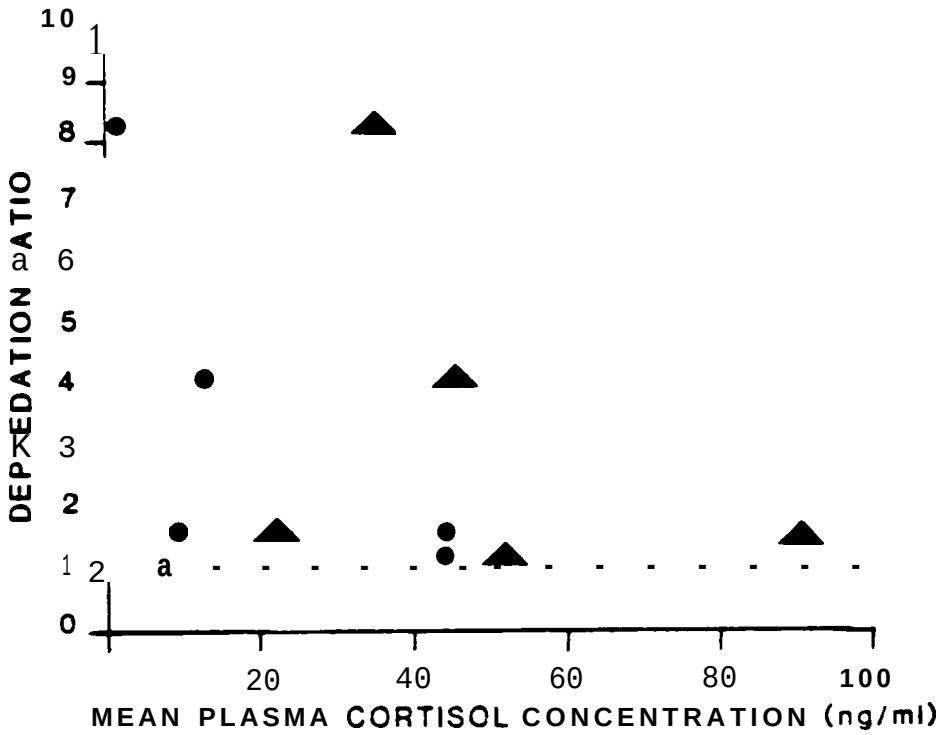


Figure 4.17. Relationship between mean plasma cortisol concentration and depredation ratios (dp) for Rapid River spring chinook and rainbow trout predators. Crowding density was held constant at 335 g/liter.

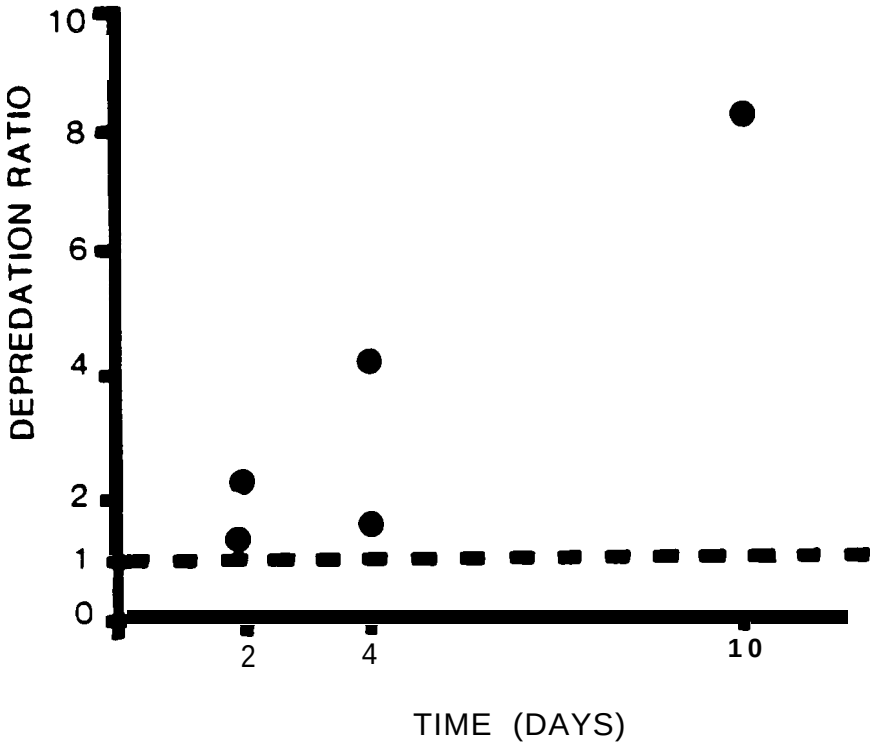


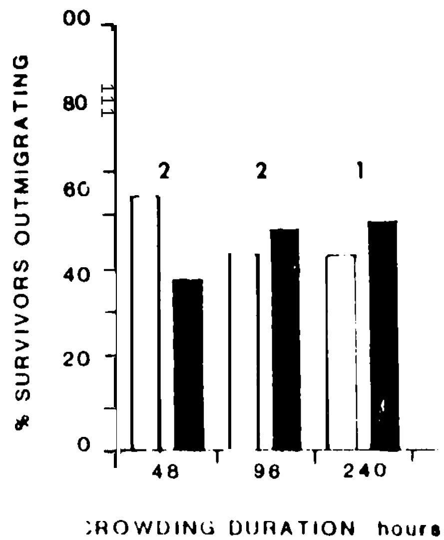
Figure 4.18. Relationship between crowding duration and depredation ratios (dp) for Rapid River spring chinook and rainbow trout predators. Crowding density was held constant at 385 g liter.

Plasma Na+ concentrations were not significantly depressed in any trial with Rapid River chinook salmon prey, regardless of crowding duration, and therefore were not correlated with prey vulnerability- Mean plasma Na+ concentrations were 134-141 meq/liter in stressed prey and 138-145 meg/liter in control prey.

Outmigration rates were similar for stressed and control fish (Fig. 4.19)‘ Stressed prey were observed occupying riffles more often than controls (Fig. 4.20) and were also more likely to be located at the surface or in contact with the substrate (Fig. 4.21).

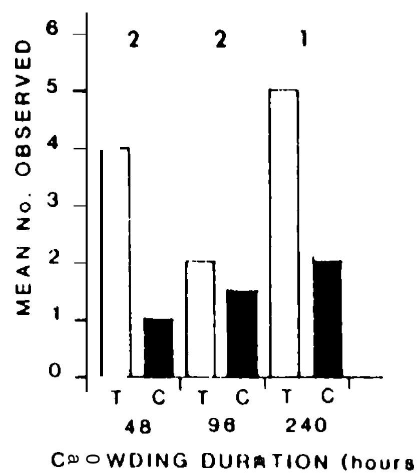
Northern Squawfish as Predators in Circular Pools

Predation on Spring Chinook Salmon, Kooskia Stock: Ten trials were completed with Kooskia chinook salmon prey and northern sguawfish predators. Observations of predator-prey interactions were precluded due to the crepuscular feeding habits of squawf ish; lighting levels were too low for observation. Crowding density of prey was held at 385 g/liter for 2, 4, and 10 days. Mean plasma cortisol concentrations were 33-237 ng/ml in crowded fish and 4-49 ng/ml in controls. Plasma cortisol concentration increased with increased crowding duration (Fig. 4.22). Prey vulnerability was related to plasma cortisol concentration ($0.025 < P < 0.05$, $r = 0.81$; Fig. 4.23) and was also correlated with crowding duration ($P = 0.05$, $r = 0.99$; Fig. 4.24). Depredation ratios climbed consistently with increased crowding duration (2-day dp = 10; 4-day dp = 1.7; 10-day dp = 2.8), but differential predation was insignificant for all paired trials. This nonsignificance does not imply that differential predation did not take place with this group of prey: with all tests pooled, survival of treatment fish was significantly less than that



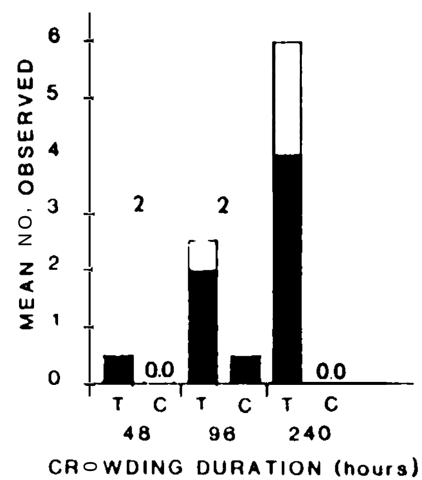
1.

Relat[illegible] out[illegible] of [illegible] [illegible] and [illegible] the [illegible] open [illegible] present crowded fish [illegible] shaded bars the [illegible] trials. [illegible] at the top of bars indicate number of [illegible] at each crowding duration.



4

Mean number of Rapid River prey types in riffle section of artificial stream channel during trials with rainbow trout [illegible] Open bars present crowded fish and shaded bars [illegible] at the control [illegible]. [illegible] at the top of bars indicate number of trials at each crowding duration.



Mean number of crowded and control Rapid River prey observed at the water surface or in contact with the channel substrate. T treatment fish; C control fish. Numbers at the top of bars indicate number of trials at each crowding duration.

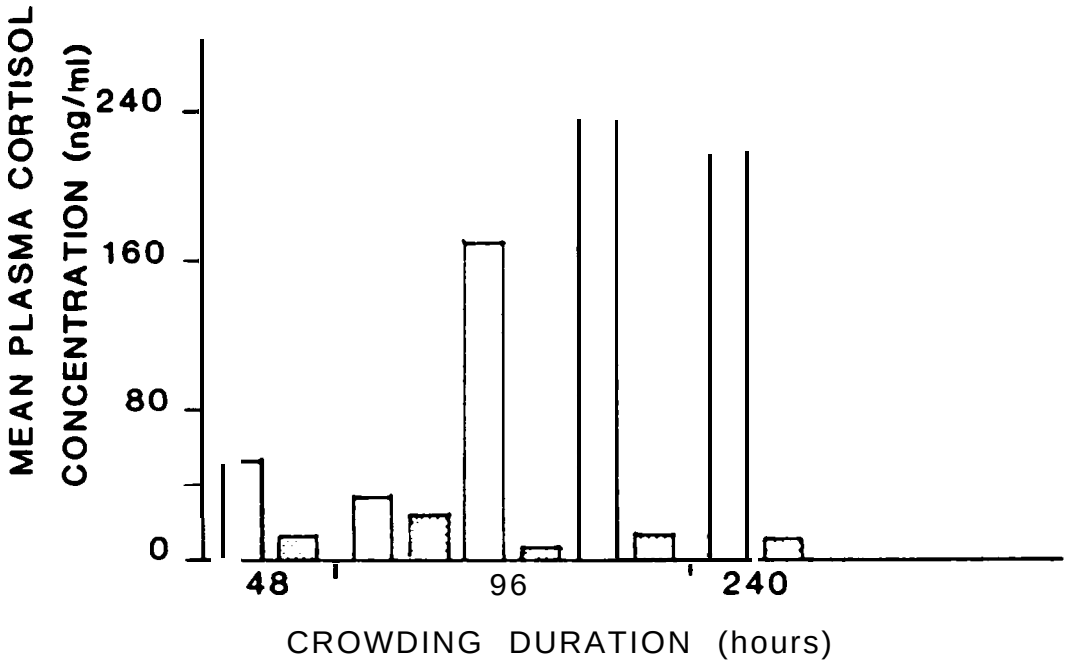


Figure 4.22. Relationshig between crowding duration and mean plasma cortisol concentration for Kooskia spring chinook salmon at introduction to circular pools containing northern squawfish predators. Open bars are means for crowded fish and shaded bars represent corresponding control means. Fish were crowded at 385 g/liter.

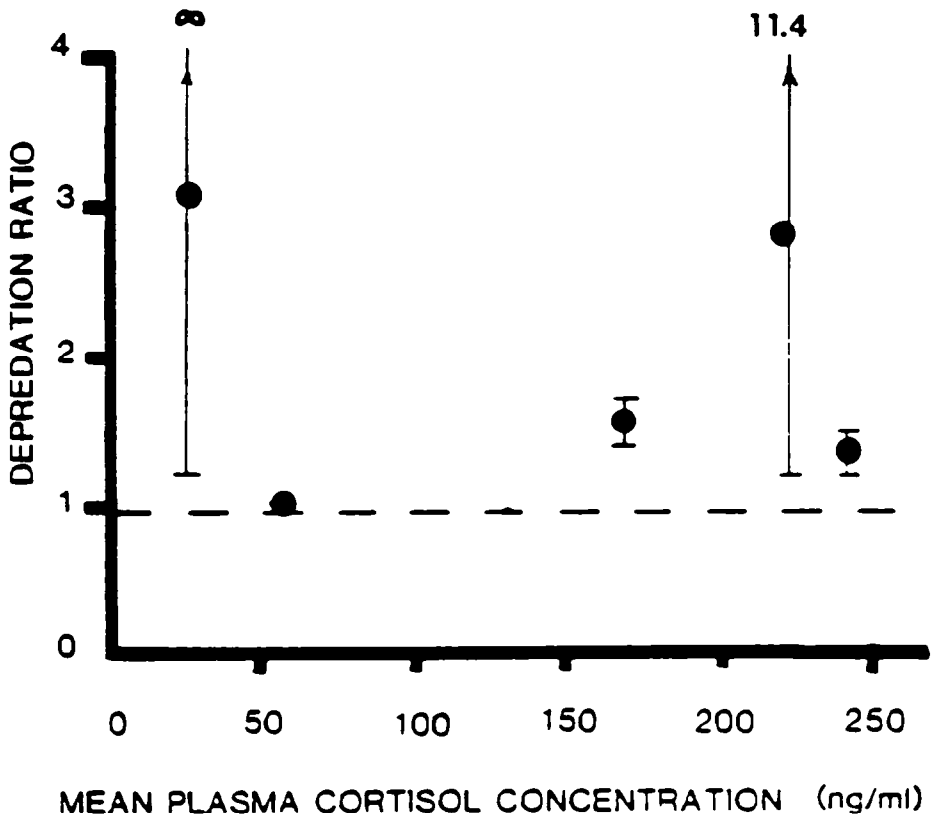


Figure 4.23. Relationship between mean plasma cortisol concentrations and depredation ratios (dp) for Kooskia spring chinook salmon and northern squawfish predators. Crowding density was held at 385 g/liter. Vertical lines indicate dp range for two simultaneous trials.

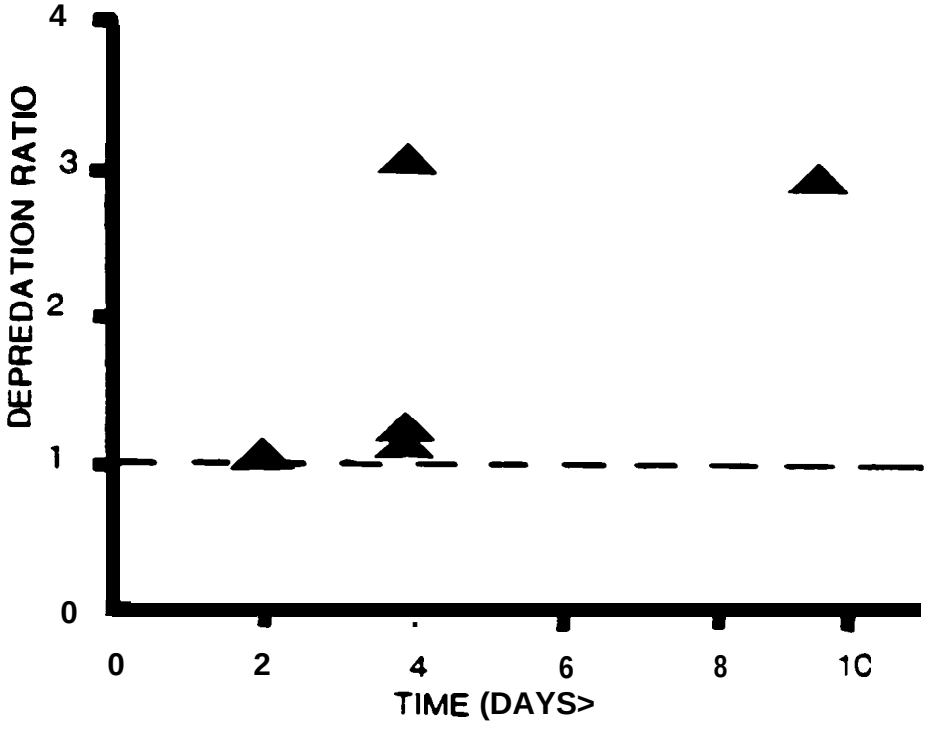


Figure 4.24. Relationship between crowding duration and depredation ratios (dp) for Rooskia spring chinook and northern squawfish predators. Crowding density was held constant at 385 g/liter.

of control fish ($0.025 < P < 0.05$). Mean plasma Na^+ concentrations were 120-140 meg/liter for crowded fish and 131-141 meg/liter for controls. Correlation between Na^+ levels and dp were insignificant, but more prey with depressed plasma Na^+ concentrations were **eaten** than were corresponding control fish.

Discussion

Vulnerability to capture by predators, as indicated by depredation ratio $(dp)_r$ increased significantly in all stocks of spring chinook salmon after they were crowded at higher densities (193 and 385 g/liter) and longer periods. Vulnerability to capture was not increased in Klickitat fall chinook crowded at a lower density (128 g/liter for 96 hours), and was significantly increased in Rapid River spring chinook salmon only after 240 hours of crowding at 385 g/liter. However, only 4 trials of 48 and 96 hours duration were completed with the Rapid River fish.

Plasma cortisol concentrations increased with increases in loading density, but did not consistently increase with increases in duration. Variation in cortisol concentrations in fish used in replicated tests **was** high. **For example,** cortisol concentrations in Klickitat **fall** chinook salmon crowded **at** 128 g/liter for 96 hours ranged from 18 to 150 ng/ml. Cortisol concentrations were also highly variable in uncrowded control fish. Other workers have **also** reported much variation in **plasma** cortisol concentrations, particularly in fish crowded for extended periods (Strange et **al**. 1978).

Predation rates were generally increased on prey with elevated plasma cortisol. Vulnerability of Little White Salmon (with smallmouth bass as predators) and Kooskia (with rainbow trout as predators) spring chinook salmon was

increased when cortisol concentrations exceeded 75-125 ng/ml. Plasma cortisol concentrations and depredation ratios were significantly correlated in these tests. The relationship between dp and cortisol concentrations was less clear in tests with Kooskia spring chinook salmon in which sguawfish were the predators, although dp was significantly elevated when all 10 tests were pooled. In another group of Kooskia spring chinook salmon (with smallmouth bass as predators), the dp ratio was significantly elevated at a plasma cortisol concentration of 100 ng/ml, but not at 65 ng/ml.

Mean plasma cortisol levels in trucked and barged chinook salmon smolts frequently range from 150 to 300 ng/ml at the time of release (Congleton et al. 1984), therefore are above the range of 75-150 ng/ml found to be associated with impaired predator avoidance ability of smolts in the present study. Releases of barged fish are made at night in mid river, a procedure that should reduce exposure to predators during the first hours of recovery. On the other hand, releases of trucked fish are usually made at one selected boat launch ramp, a procedure that could attract predacious fishes to the site.

We believe that increased vulnerability of crowded Kooskia spring chinook salmon to predation was to some extent a consequence of pathological changes in the eyes. The lenses of the eyes of many of these fish were cloudy or degenerated after 240 hours of crowding at 385 g/liter. The etiology of these change is unknown. The eyes of chinook salmon of the Little White Salmon and Kooskia strains did not seem to be affected, perhaps because the maximum crowding duration in trials with these groups was 96 rather than 240 hours.

Test conditions were not identical for all stocks of chinook salmon used in predator-prey trials, limiting the validity of between-stock comparisons. Several test habitats and prdeatory species were used, and the degree of smoltification of prey stocks also differed. Smoltification strongly increases the physiological response of spring chinook salmon (and presumably all anadromous salmonids) to crowding stress. Spring chinook salmon smolts sampled at Dworshak NFH had higher baseline cortisol titers (20-40 w/ml1 than did parr (Cl0 w/ml), and cortisol concentrations were higher in crowded smolts than in crowded parr (see section 2 of this report).

Only two groups of prey were tested during the normal period of smoltification and outmigration (spring to early summer): the Little White Salmon (smallmouth bass as predators) and Kooskia (trout as predators). Little White Salmon chinook outmigrated from an experimental migrational channel at the station from early February (when trials were begun) until May, when trials were discontinued. Kooskia chinook salmon had the external appearance of smolts and actively outmigrated from the experimental stream channel when trials were begun in early June, with migrational tendency generally decreasing as the summer progressed. Maximum mean plasma cortisol concentrations after crowding of fish from these stocks exceeded 200 ng/ml. Mean cortisol concentrations also exceeded 200 ng/ml in fish from the Kooskia stock after crowding in early fall tests. In contrast, peak cortisol concentrations in Kooskia chinook salmon subjected to crowding in mid winter, and in Rapid River chinook salmon subjected to crowding in fall, did not exceed 100 ng/ml.

Necessarily artificial conditions in the test habitats may have reduced differential predation on stressed (crowded) prey. Test conditions could have affected

perdation on stressed prey during each of the three phases of the predation process described by Barns (1967):

1. Discoverv. Two groups of prey can be available simultaneously but discovered at different rates, resulting in differential predation. Due to the small size of the pools used in trials with bass and sguawfish, stressed and control prey were available more or less equally at all times. In stream channel trials, however, the tendency of stressed chinook salmon to seek refuge in the shallow riffle sections of the stream undoubtedly reduced the rate at which they were discovered by the predators. Although riffle areas were a haven for prey in these trials, prey entering shallow water under natural conditions would be vulnerable to attack by avian and mammalian predators.

2. Attack. After discovery, predators may attack prey at random or select individuals discriminately. Shaw (1978) and Neil1 and Cullen (1974) both reported that predatory fishes typically isolated individual prey from schools before they attacked. This behavior was observed repeatedly in both smallmouth bass and rainbow trout in the present study. Inhibition of predatory attacks on tightly schooled prey may have protected stressed prey in our tests. Schooling could be expected to mask subtle behavioral differences between control and stressed prey, such as decreased reaction distance and increased reaction time to approaching predators, or decreased peak swimming speed. Impaired stressed prey may have been able to school with unimpaired control fish more successfully in our tests than would have been possible in the natural environment, because the dimensions of the test

habitats used limited mobility of the school to only a few feet in any direction.

3. Canture. Schooling has been shown to enhance the ability of fish to respond to environmental stimuli (Ralson 1981), so stressed chinook salmon may have benefited from the greater vigilance and unimpaired response of their companions in the school. Under test conditions, stressed prey formed mixed schools with unstressed prey and presumably gained some protection from predators through this **association.** All chinook salmon released into the Columbia River after collection and transportation would be stressed, and would not immediately **have** the opportunity to school with unstressed fish.

Summary

1. Vulnerability to predator attack **was** increased in spring chinook salmon after they were crowded at densities of 193 and 385 g/liter for 96-240 hours. Vulnerability **was** not significantly increased in fish crowded at lower densities or for shorter intervals.

2. The relation between increased predation mortality and plasma cortisol was highly variable, but predation rates and plasma cortisol concentrations were significantly correlated in trials with three stocks of chinook salmon. Fish of two other stocks were either used in only a few tests or were not tested after exposure to high densities.

3. Crowding elicited **a** larger increase in plasma cortisol and **a** greater increase in vulnerability to predators in smolts of chinook salmon than in Parr.

4. Although plasma Na^+ concentrations and depredation ratios were not significantly correlated (prey with mean Na^+ concentrations within the range observed for controls were often highly vulnerable), prey with depressed plasma Na^+ concentrations were more vulnerable to predation than were corresponding controls, in all tests.

5. In several groups of chinook salmon tested under different conditions, predation mortality was higher in fish with plasma cortisol concentrations of 75-150 ng/ml and above than in fish with lower concentrations, Cortisol concentrations often exceed 150-250 ng/ml in trucked and barged fish. Repeated releases of trucked fish at one site could attract predators and result in significant predation mortality. Predation on barged fish after release is less likely to be of significance because repeated releases are not made in the same location.

5. BACTERIAL KIDNEY DISEASE AS RELATED TO STRESS AND TRANSPORTATION OF CHINOOK SALMON SMOLTS

Bacterial kidney disease (BKD) is present in varying degrees in both wild and hatchery salmon smolts in Idaho and may be stress mediated. In 1982, we found that salmon passing Lower Granite and Little Goose dams were stressed (elevated plasma cortisol and glucose concentrations) and hypothesized that they might be more vulnerable to BKD than unstressed fish.

In 1982 and 1983, we tested chinook salmon smolts transported from hatcheries to MFS in Puget Sound and held up to 173 days in sea water to see if mortality due to BKD would increase following the stresses of transportation. If some groups transported to MFS survived well in sea water, then additional testing of fish collected at the dams would be warranted. Salmon change behaviorally and physiologically once they begin migrating seaward, and thus the stress response in fish taken from a hatchery may not be exactly the same as that of fish migrating seaward and collected at the dams.

Methods and Materials

1982 Experiments

Three groups of age 1 spring chinook salmon (Dworshak NFH "srnallv@ and "large" and Rapid River Hatchery) and one group of age 0 fall chinook salmon (Hagerman NFH) were transported to MFS on Puget Sound, acclimated to sea water, and held for 3-6 months. The incidence of BKD in each hatchery population was estimated by the direct fluorescent antibody technique (DFAT). After transport, the fish were routinely held for 2 days in fresh water, followed by 2 days

at loo/o0 salinity and 2 days at 20°/oo salinity before introduction to full strength (28'/00) sea water. The effect of delayed entry into sea water was examined by holding some groups in fresh water for a month or more after transport to MFS.

1983 Experiments

Yearling and age 0 spring chinook salmon and age 0 fall chinook salmon were taken from Dworshak, Kooskia, and Hagerman NFHs to compare smolts of various stocks and with different rearing regimes, ages, and mortality rates in the hatcheries (Table 5.1). We evaluated the effects of transport medium (fresh water versus loo/o0 sea water), medium at release site (fresh water versus loo/o0 sea water as in the estuary), transport stress level (low versus purposely increased), and administration of erythromycin in the feed before transport (treated feed versus untreated feed) by monitoring subsequent survival of fish in tanks supplied with sea water at MFS.

The response of chinook salmon smolts to the various test conditions or treatments was evaluated in terms of mortality, stress response (as measured by plasma cortisol) and BKD infection rates. Mortality and BKD infection rates in each group of salmon tested were monitored while in the hatchery and during 78 to 130-day periods of rearing in sea water. Plasma samples were taken from smolts at the hatchery before they were loaded, 2 hours after loading, upon arrival at MFS (14 hours after loading), and then 2, 24, and 96 hours after being placed in tanks at MFS to assess stress levels (Fig. 5.1). Procedures for blood sampling and cortisol analyses were as described in preceding sections of this report.

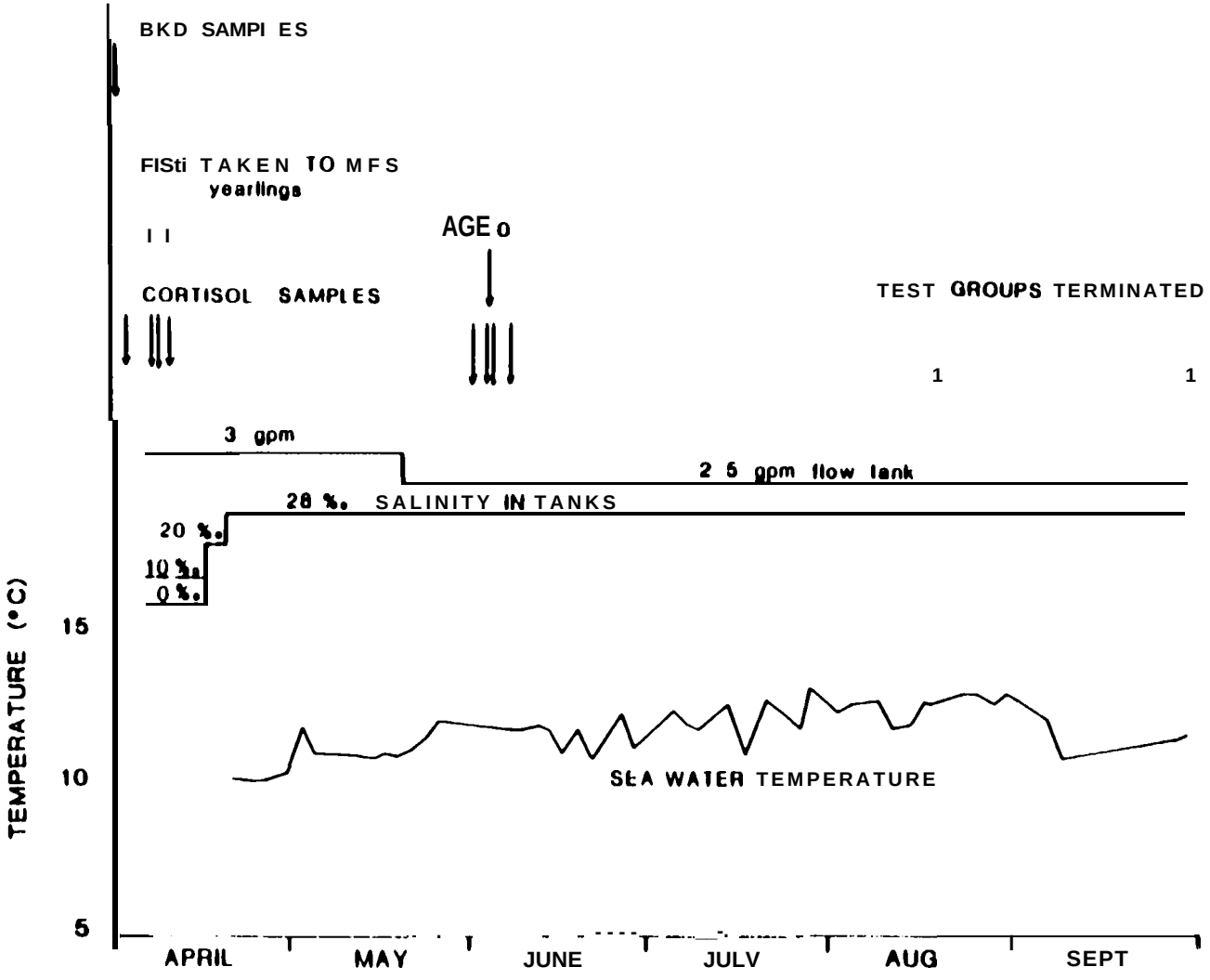


Figure 5.1. Approximate dates on which samples were taken for bacterial kidney disease and cortisol analyses, fish transported from hatcheries to Marrowstone Field Station, flow rates of sea water in tanks, salinity of sea water in tanks, dnd temperature of sea water, 1983.

Chinook salmon of five stocks (Leavenworth, Kooskia, Rapid River and Little White Salmon spring chinook salmon, and Snake River fall chinook salmon) reared at three hatcheries (Kooskia, **Hagerman** and Dworshak) were tested (Table 5.1). Fish were taken to MFS in early April (yearling spring chinook salmon) and early June (age 0 spring and fall chinook salmon). Rearing was terminated for most groups on 20-21 August after 78 (age 0 fish) and 131 (yearlings) days.

At the hatchery, fish were crowded in the raceway to enable us to obtain a representative sample. A dip net with **a** sanctuary **bag** to keep the fish in water **was** used to transfer fish from the raceway to a tank truck. Each test group was transported in a separate, isolated section of **a** transport tank at about 66 g/liter (0.55 pounds/gallon). Fish were unloaded from the tank truck at MFS using the same net (with sanctuary bag) for light stress groups and with **a** regular net for groups that were purposely stressed. Purposely stressed fish were suspended in a net out of water for 30 seconds; at the end of this period, most fish had ceased struggling.

Kooskia stock yearlings in one raceway at Kooskia NFH were fed Oregon Moist Pellets containing erythromycin (6.6 g/pound of feed) for 21 days in late March and early April. Fish in adjacent raceways were fed regular (unmedicated) feed.

Water for fish transported in fresh water **was** obtained at the hatchery. Dilute **sea** water (lO"/oo) for transport **was** obtained by partly filling the tanks with sea water at MFS and then adding fresh water at the hatchery to obtain the desired dilution.

Table 5. Variables tested on each of six groups of chinook salmon taken to Marrowstone Field Station in 1983.

Variables	Stocks of fish					
	Kooskia springs reared at Kooskia NFH (age 1)	Little White Salmon springs reared at Dworshak NFH (age 1)	Leavenworth springs reared at Kooskia NFH (age 0)	Kooskia springs reared at Hagerman NFH (age 0)	Rapid River springs reared at Hagerman NFH (age 0)	Snake River fall chinook reared at Hagerman NFH (age 0)
Erythromycin feed before transport						
Medicated	X		-	-	-	-
Unmedicated	X	X	X	X	X	X
Transport medium						
Freshwater	X	-	X	X	X	X
Sea water (10 o/oo)	X	X		-	-	-
Stress when unloaded at MFS						
Stressed	X	-	X	X	X	X
Unstressed	X	X	X	X	X	X
Recovery medium at MFS						
Fresh water	X		X	X	X	X
Sea water (10 o/oo)	X	X	-	-	-	-

After their arrival at MFS, some groups of fish were placed in fresh water and some in dilute sea water (lO"/oo) for 6-7 days to recover from transportation. After the recovery period, salinity was increased in three steps (10, 2O, 28O/oo) at three 3-day intervals. None of the chinook salmon showed evidence of osmoregulatory problems with this transition to sea water.

At MFS, fish were fed Oregon Moist Pellets at a rate of l.l%-2.3% of body weight per day, depending on their size. Samples of fish were weighed and measured at the start and end of rearing in sea water.

At MFS, yearling chinook salmon from Kooskia NFH were held in 5-foot circular tanks (1300 liters), 4-foot circular tanks (600 liters), and 2 x 2 x 6-foot troughs (577 liters) at different density indices (Fig. 5.2). (Density index = pounds of fish divided by the product of tank volume x fish length.) End-of-test loadings with 95 fish per tank were highest in 4-foot circular tanks and troughs (14 g/liter, density index 0.10) and lowest in the 5-foot circular tanks(6.3 g/liter, density index of 0.05). At the hatchery, fish were loaded at 16.5 g/liter and a density index of 0.17 (Fig. 5.2). Water flows in the tanks were 3 gpm for the first 5 weeks, and 2.5 gpm thereafter. At 2.5 gpm, the water exchange rate was 0.94 and 0.44 times/hour for the 4-foot and 5-foot tanks, respectively.

Results

1982 Experiments

Although survival of all groups was high during the first few weeks in sea water, survival was low (15%-67%) after 130 days (Table 5.2). Fluorescent antibody tests indicated a high incidence of BKD (59% and 70%) in the two

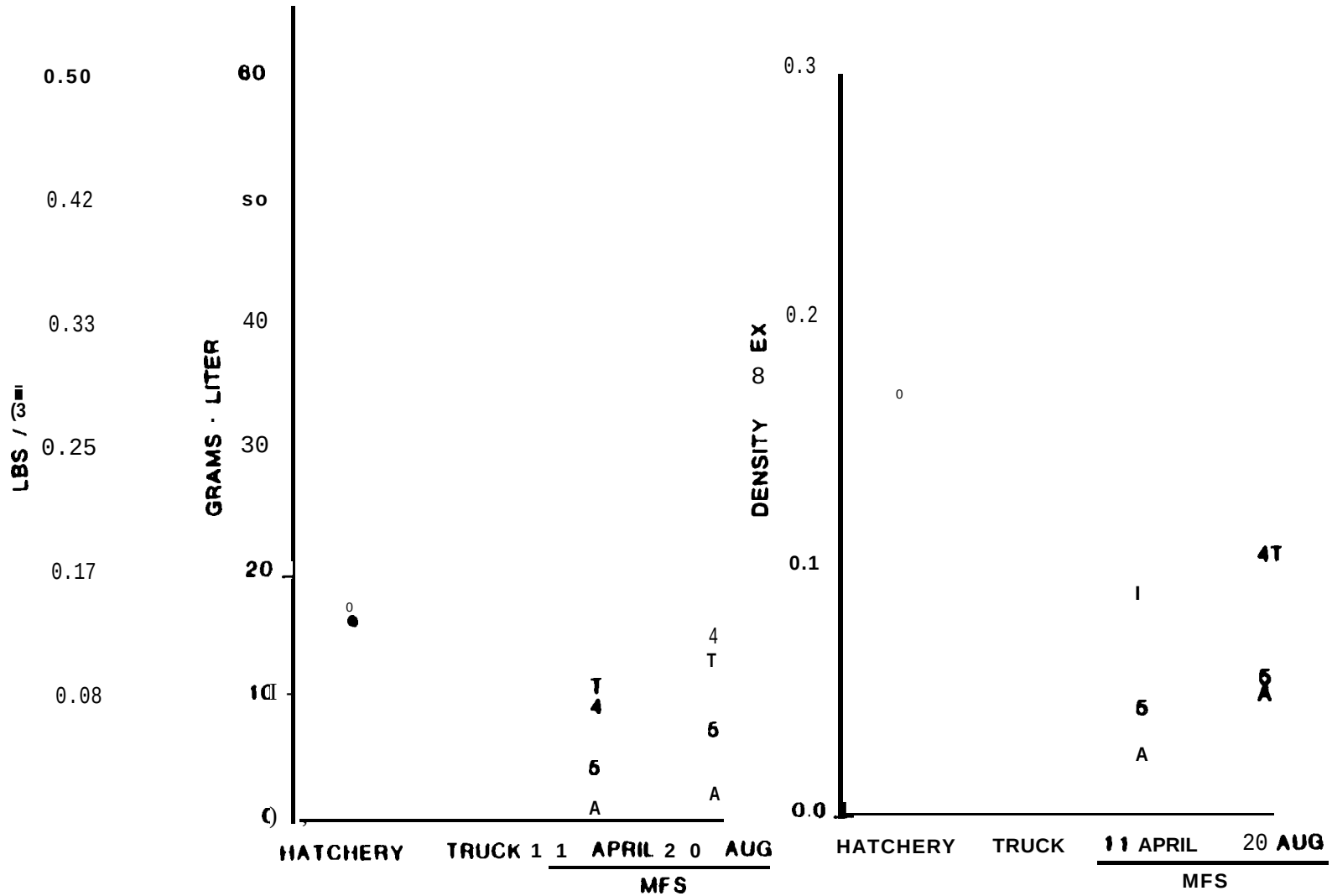


Figure 5.2. Loading rates and density indices for spring chinook salmon reared in Kooskia NFH raceways, during truck transport, and at start (11 April) and end (20 August) of rearing at Marrowstone FS in five-foot circular tanks (5), four-foot circular tanks (4), wooden trough (T), and 30-gallon aquaria (A).

groups from Dworhsak NFH, and a very low indicence (0%-l%) in Hagerman age 0 fall chinook salmon and Rapid River chinook salmon (Table 5.2). Surprisingly, the group with the highest intitial incidence of BKD (Dworshak NFH V1small*9 chinook salmon) survived in sea water at a higher rate than other groups. Rapid River chinook salmon, with an initial BKD-positive score of only l%, survived in sea water at a substantially lower rate than other groups (Table 5.2: Fig. 5.3). Despite their initial BKD score, the Rapid River group tested 70% positive for BKD after 10 weeks in sea water.

1983 Experiments

Yearling spring chinook salmon smolts reared in the raceways at Kooskia NFH survived at consistently high rates in sea water at MFS regardless of feed medication, transport media, stress when unloaded from tank truck, or recovery media (Table 5.3). Survival of fish held in 5-foot tanks averaged 96% for the 131-day rearing period. Fish held in 4-foot circular tanks or 6-foot troughs survived at slightly lower rates (85% and 87%; Fig. 5.4).

Only O.l%-0.2% of the Kooskia yearlings died during the last three months of rearing at the hatchery, whereas, 4% of the fish in 5-foot circular tanks died in 4.4 months of rearing in sea water at MFS. The loss rate was thus appreciably higher in sea water, but still relatively low compared with rates in other groups of chinook salmon tested.

Stress caused by suspending fish out of water in a net for 30 seconds did not increase mortality in Kooskia yearlings (Table 5.3) or age 0 smolts (Table 5.4). Mortality in the Kooskia yearling salmon was low with or without the extra stress: mortality of age 0 chinook salmon

Table 5.2. BKD incidence and mortality in chinook salmon groups held in sea water for 130 days (April-August 1982).

Source/age	No. Groups	% BKD in hatchery (April)	% Survival 21 days in sea water	% Survival 130 days in sea water
DNFH "large" Age 1	3	59	95	50
DNFH "small" Age 1	2	70	91	67
DNFH "small"[a] Age 1	1	70	94	33
Hagerman NFH Age 0 fall chinook	4	0	95	63
Rapid River FH[a] Age 1	1	1	95	15

[a] Maintained 41 days in fresh water before introduction to sea water. Mortality in fresh water was minimal (<1%) in Rapid River group and 17% in DNFH "small" group.

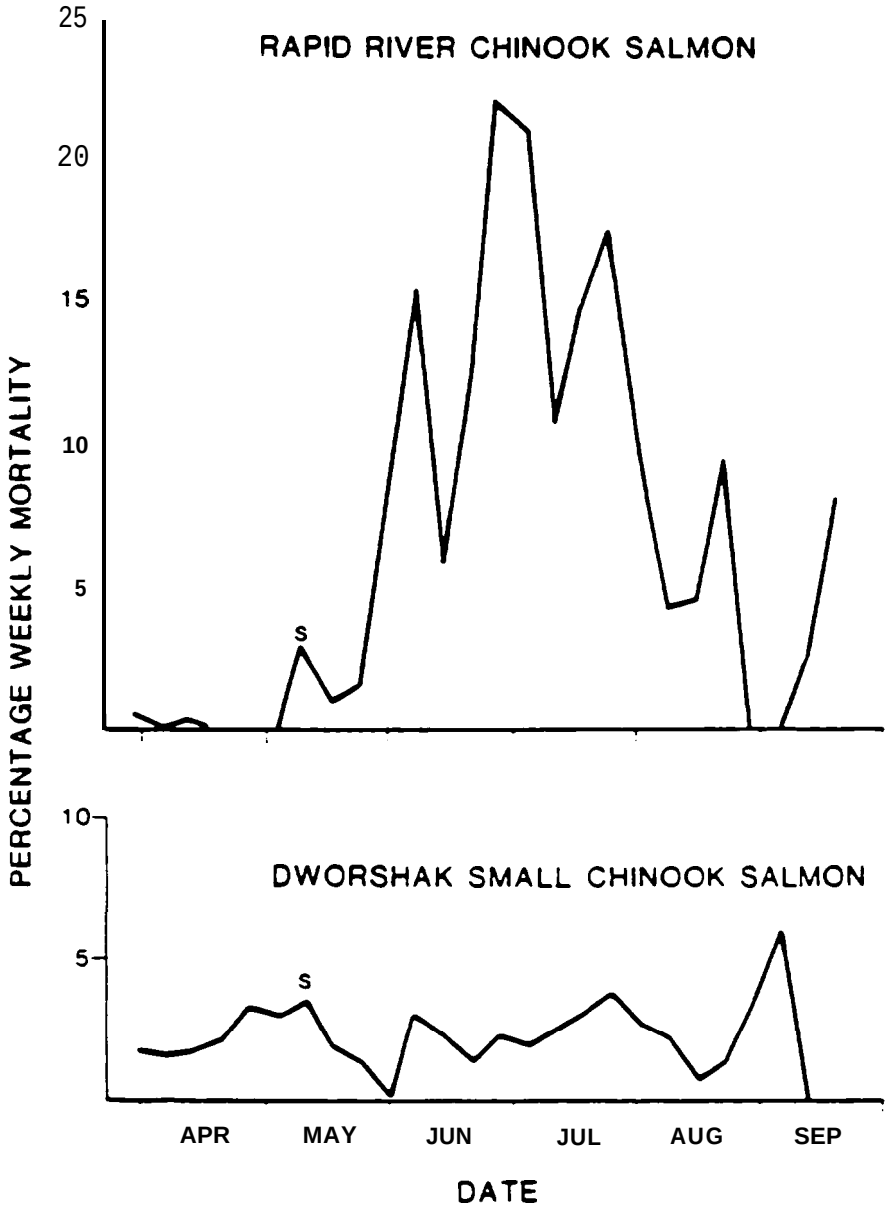


Figure 5.3. Percentage weekly mortality in Rapid River Hatchery and Dworshak Hatchery "small" chinook salmon reared in sea water for 1.5 months. S = Sate of acclimation to sea water.

Table 5.3. Survival rates and BKD incidence in age 1 spring chinook salmon during last three months at Dworshak NFH and Kooskia NFH, and survival while at Marrowstone Field Station 9-11 April to 20-21 August 1983 for most groups

Group description	Survival rate in hatchery %	BKD incidence at hatchery %	Survival (%) at Marrowstone			
			39 days	131-133 days		171-173 days
				Circular tanks	Troughs	
Kooskia stock reared at Kooskia NFH						
Medicated erythromycin feed	99.9	45				
Transported in fresh water:						
Stressed at MFS						
Recovery in fresh water			-	98.0	86.4	
Recovery in sea water			-	98.0		-
Not stressed at MFS						
Recovery in fresh water			-	93.3		
Recovery in sea water			-	35.1		39.3
Transported in 10° oo sea water:						
Stressed at MFS						
Recovery in fresh water				96.2	90.0	
Recovery in sea water				99.0	91.8	91.4
Not stressed at MFS						
Recovery in fresh water			100.0[a]	-	91.3	
Recovery in sea water				90.5[a]	35.3	
Without medicated feed	99.8	45				
Transported in fresh water:						
Stressed at MFS						
Recovery in fresh water				95.2	33.3	
Recovery in sea water				97.1	88.0	
Not stressed at MFS						
Recovery in fresh water				95.2	91.0	
Recovery in sea water				93.1	93.7	
Transported in 10° oo sea water:						
Not stressed at MFS						
Recovery in fresh water				82.9[a]	66.0	
Recovery in sea water				93.0[a]	67.2	
Little White Salmon NFH stock reared at Dworshak NFH						
Unmedicated feed	39.9	50				
Transported in 10° oo sea water:						
Not stressed at MFS						
Recovery in 10° oo sea water						
Large fish (229 mm):			84.7[a]	-		
Small fish (142 mm):				65.8[a]		

[a] Four-foot diameter circular tanks. All others were five-foot diameter.

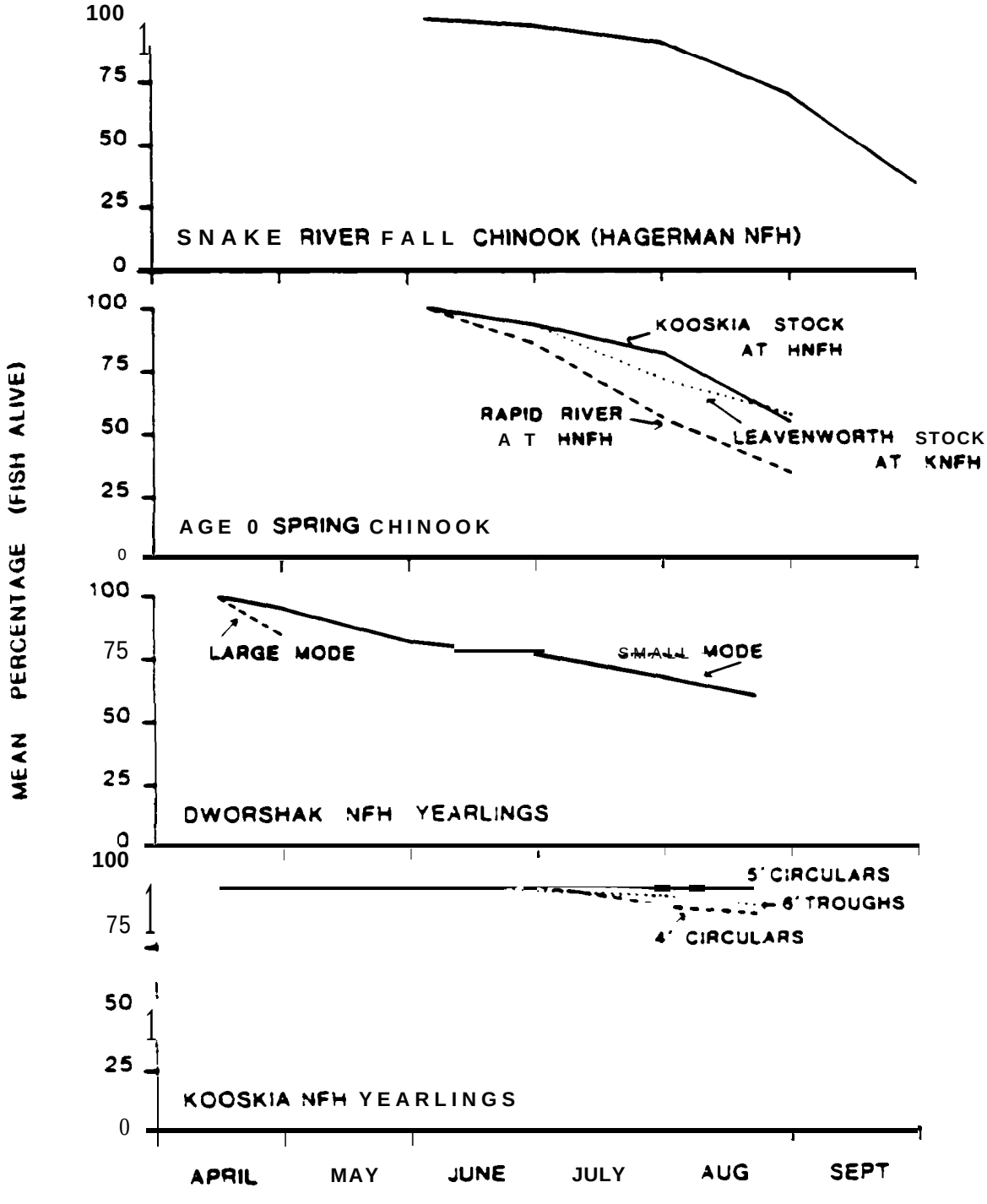


Figure 5.1. Survival rates of spring and fall chinook salmon smolts in tanks supplied with sea water at Marrowstone Field Station, 1983.

Table 5.4. Bacterial kidney disease (BKD) incidence at hatcher) and survival rates at Marrowstone Field Station of age 0 spring and fall chinook salmon in 1983. All but Rapid River stock fish were held in 4-foot circular tanks for 78 days. Rapid River fish were held in 6-foot long troughs.

Group of fish	BKD incidence (%)	Survival (%)
Kooskia stock reared at Hagerman NFH	56.0	
Stressed		54.1
Unstressed		56.0
Rapid River stock reared at Hagerman NFH	72.0	
Stressed		32.1
Unstressed		37.:
Leavenworth stock reared at Kooskia NFH	11.5	
Stressed		64.2
Unstressed		48.6
Snake River fall chinook reared at Hagerman NFH	10.0	
Stressed		75.0[a]
Unstressed		63.4[a]

[a] These groups were held an additional 40 days and had survivals of 54.85 and 16.8% after 118 days of rearing.

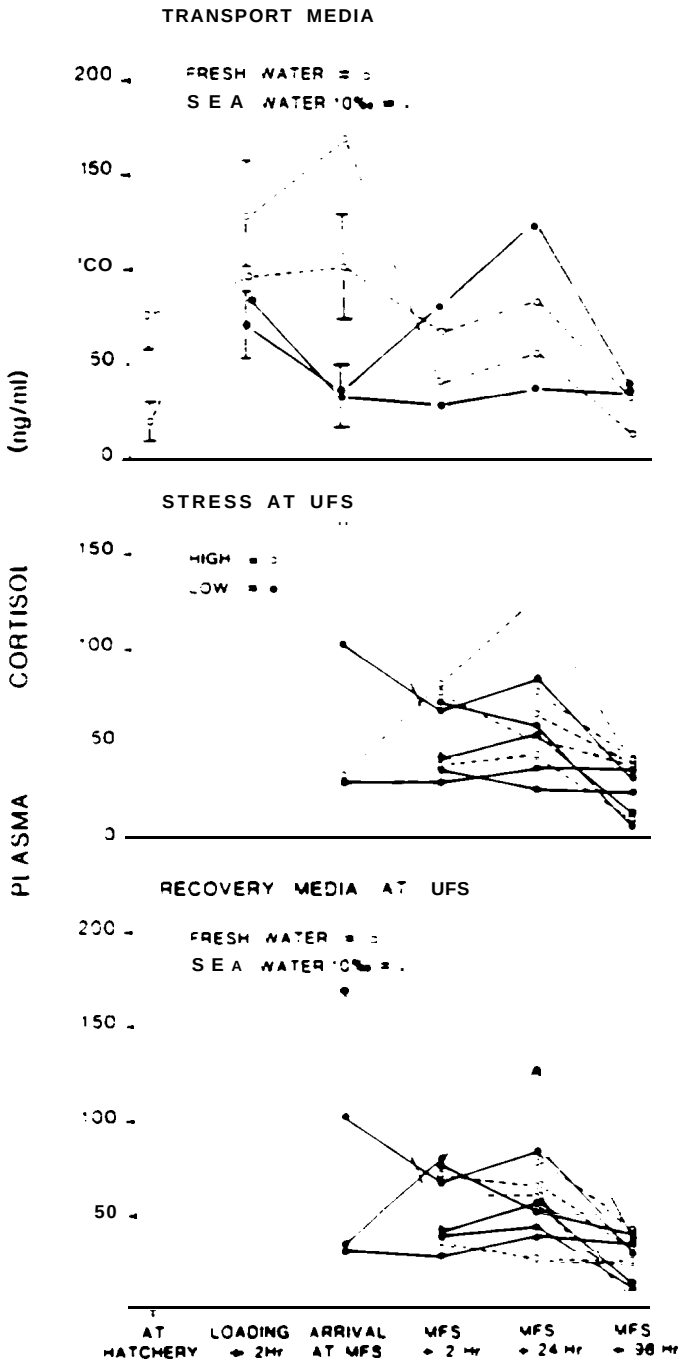
smolts was high, but not higher in the groups exposed to extra stress.

Among yearling spring chinook salmon of the Little White Salmon stock, chronic losses (10%) during the final 3 months of rearing at Dworshak NFH were typical of losses in fish suffering from BKD (Table 5.3). Fish of this group had a bimodal length distribution and those taken to MFS in 1983 were divided into two modes: a large mode (229 mm mean total length) and a small mode (143 mm). At MFS, 15% of the large-mode fish died in 39 days of rearing, before a pump malfunction resulted in the suffocation of the remaining fish. Among small-mode fish kept 131 days in sea water, 34% died (Table 5.3). Mortality was relatively high in this group of fish despite transportation and recovery in dilute sea water.

None of the groups of age 0 spring chinook salmon taken to MFS in 1983 survived at acceptable rates in sea water (Table 5.4). Since the two groups reared at Hagerman NFH before transport to MFS had incurred losses from BKD while at the hatchery, we were not surprised when a large proportion of the fish died in sea water. However, age 0 smolts produced at Kooskia NFH incurred minor losses while at the hatchery (1.1% in last 74 days), but nevertheless died at a high rate (45% in 78 days) at MFS (Table 5.4). Virtually all the fish that died had typical signs of BKD.

Plasma Cortisol

Two groups of yearling spring chinook salmon at Kooskia NFH had significantly different mean plasma cortisol concentrations of 22 and 77 ng/ml when sampled at the hatchery on April 4 (Fig. 5.5). The group with the lower mean concentration had been medicated with erythromycin during the previous 3 weeks: these concentrations were on



ure 5.5. Plasma cortisol concentrations of yearling spring chinook salmon smolts before, during, and after transport from Kooskia NFH to Marrowstone PS in 1983. Bars in upper figure are 95% confidence limits for means; confidence limits were of similar magnitude for datum points in lower figures, but are not shown to maintain legibility.

the low end of the range we would expect in smolts. The fish with the higher cortisol concentration had been fed regular unmedicated feed. Two hours after the fish were loaded into the transport tank, and again upon arrival at MFS, cortisol concentrations in both groups of fish were higher than before transport, but not significantly different from each other (Fig. 5.5).

In Kooskia yearling salmon transported in dilute sea water, plasma cortisol concentrations 2 hours after loading into the transport tank did not differ from those in fish transported in fresh water (Fig. 5.5). After the fish had been in transport for 14-16 hours, cortisol concentrations in those transported in dilute sea water had declined back to pre-loading levels and were significantly lower than concentrations in fish transported in fresh water. Plasma cortisol concentrations in the fish during the first 96 hours in tanks at MFS were not clearly related to transport media, stress when unloaded, or recovery media (Fig. 5.5). After 96 hours in the tanks at MFS, cortisol concentrations were near baseline levels (less than 50 ng/ml) in all groups.

In yearling spring chinook salmon reared at Dworshak NFH, plasma cortisol concentrations were similar to those in yearlings from Kooskia NFH before, during and after transport to MFS (Fig. 5.6). Concentrations were less than 50 ng/ml before the fish were loaded into the transport tank but increased to over 100 mg/ml 2 hours after loading. Upon arrival at MFS, concentrations were still elevated (150 ng/ml) in fish of the large mode, but were near the pre-loading levels in fish of the small mode, similar to those of Kooskia yearlings transported in dilute sea water (Figs. 5.5 and 5.6). After the fish had been at MFS for 96 hours, the plasma cortisol concentrations were near pre-transport levels.

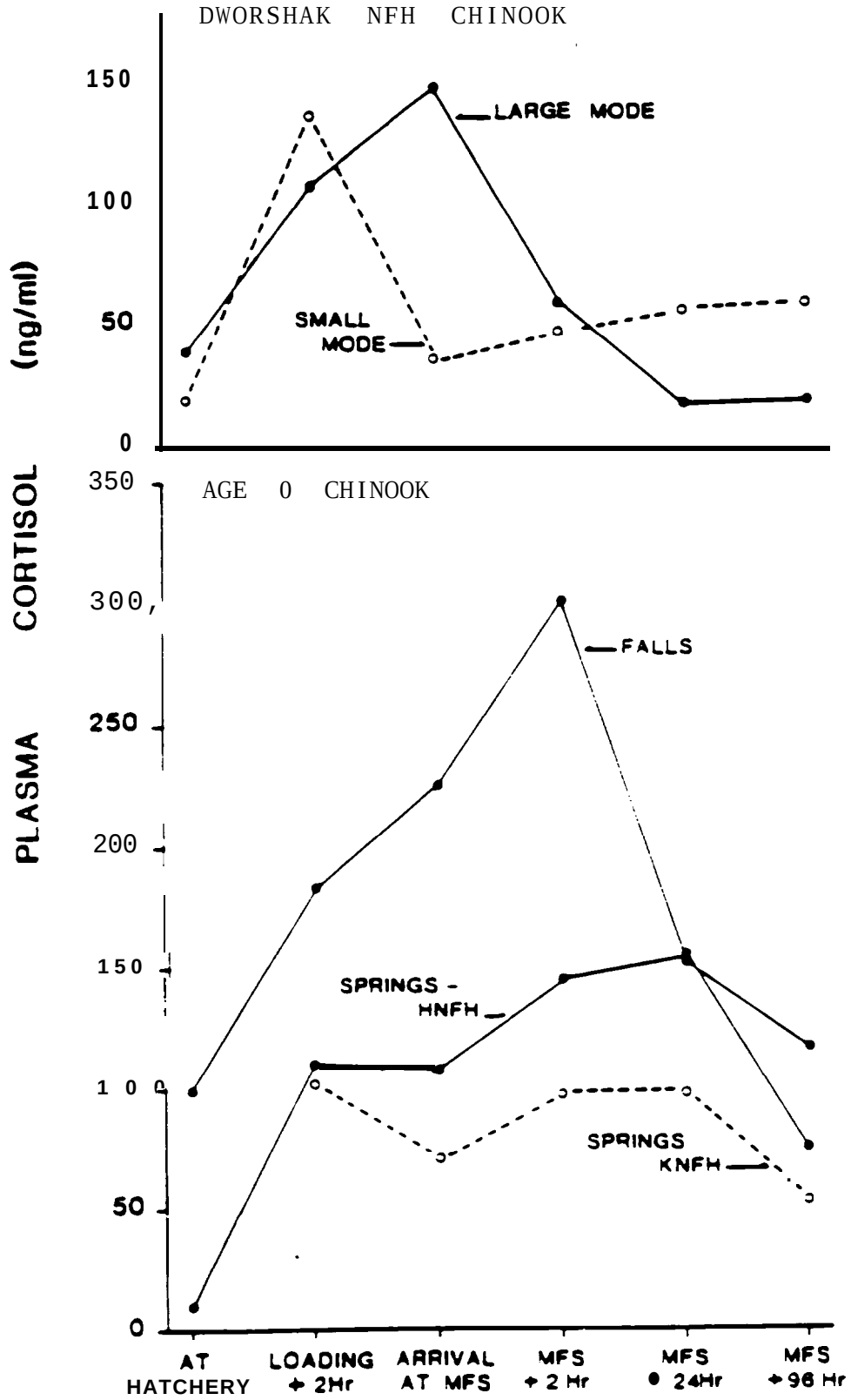


Figure 5.6. Plasma cortisol concentrations of yearling chinook salmon smolts from Dworshak NFH and age 0 spring and fall chinook salmon smolts from Kooskia and Hagerman NFHs before, during, and after transport to Marrowstone FS, 1993.

In age 0 spring chinook salmon smolts of Leavenworth stock reared at Kooskia NFH, and smolts of Kooskia stock reared at Hagerman NFH, plasma cortisol concentrations before, during, and after transport differed in some respects from those in yearling smolts (Fig. 5.6). Pre-loading concentrations of cortisol were low (20 ng/ml), as in yearlings, and increased during transport in fresh water. In age 0 spring chinook salmon smolts, the concentrations of cortisol were highest (100 and 155 ng/ml) after the fish were stressed in a net and while they were recovering in fresh water. After the fish were in tanks at MFS for 96 hours, cortisol concentrations had declined to 55 and 75 ng/ml.

In age 0 fall chinook salmon smolts raised at Hagerman NFH, plasma cortisol concentrations were relatively high (100 ng/ml) before the fish were loaded in the transport tank, and were the highest for any group 2 hours after loading (Fig. 5.6). Cortisol concentrations had increased still further by the time the fish arrived at MFS and were higher yet (302 ng/ml) 2 hours after being stressed in the net. After the fish had been moved to MFS, cortisol concentrations had dropped to 154 ng/ml by 24 hours and were nearly down to pre-transport levels after 96 hours.

Fall chinook salmon and age 0 spring chinook salmon raised at Hagerman NFH were on the transport truck 24 hours longer than fish transported from Kooskia and Dworshak NFHs. At all times, plasma cortisol concentrations were higher in the fall chinook salmon than in any of the spring chinook salmon groups.

Bacterial Kidney Disease

Survival of yearling and age 0 chinook salmon smolts at MFS in 1983 was not correlated with BKD incidence as determined by DFAT. Of the yearling spring chinook salmon smolts sampled at Kooskia NFH in mid March, 45% were DFAT-positive, yet survival in sea water at MFS was high (>90%; Table 5.3). Yearling smolts from Dworshak NFH also had a high incidence of BKD (50% DFAT-positive), but survival in sea water was not high (65%; Table 5.3).

None of the age 0 salmon smolts survived at a high rate in sea water at MFS, despite the relatively low BKD incidence of some groups. Among the age 0 smolts sampled at Kooskia NFH in May 1983, only 11.5% were DFAT-positive for BKD, but survival at MFS was only 49%-64% during 78 days of rearing (Table 5.4).

Kooskia stock chinook salmon reared at Hagerman NFH and taken to MFS in 1983 were from parents that were DFAT-positive for BKD at time of spawning but only lightly infected. While at Hagerman NFH, this group had losses from BKD early in the rearing period (l%-4% weekly in February); however, the losses then decreased to negligible levels in April and May. When these fish were sampled in March 1983, the BKD incidence was 56%. At MFS, only 55% of the fish sunrived during the 78 days of rearing in sea water (Table 5.4).

In Rapid River stock chinook salmon reared at Hagerman NFH and taken to MFS in 1983, losses from BKD were high in February and March while at the hatchery. In March, 72% of the fish sampled at the hatchery were DFAT-positive for BKD. The BKD infection rate of their parents was unknown. Only 32%-37% of these smolts survived the 78-day rearing period at MFS (Table 5.4).

The BKD incidence in fall chinook salmon reared at Hager-man NFH in 1982-1983 was relatively low (DFAT-positive) when the fish were sampled in June. At MFS, 63%-75% of these smolts survived during the 7a-day rearing period, but only 17% and 55% had survived after an additional 40 days of rearing (Table 5.4).

Discussion

Survival of yearling and age 0 chinook salmon smolts during 78-131 days of rearing in sea water at MFS correlated best with survival rate while at the hatchery. In yearling salmon reared at Kooskia NFH, mortality was low at the hatchery and also low in sea water (Table 5.1). Sunrival of Kooskia yearlings was high irrespective of whether the feed was medicated and irrespective of transport media, extra stress, or recovery media. The fish were of good quality and withstood conditions encountered during transport and rearing in sea water.

Other groups of yearling and age 0 salmon were of lesser quality, as judged by performance while in the hatchery, and their survival rates were relatively low. Stresses of transport to MFS or rearing in sea water increased mortality of these fish. The imposition of extra stress upon arrival of the fish at MFS (suspension in net for 30 seconds) did not increase mortality.

Summary

1. Survival of chinook salmon smolts during extended rearing in sea water correlated well with survival during rearing in fresh water.

2. Stresses associated with transport of fish to MFS and rearing in sea water did not cause increased mortality

in good quality smolts, but did increase mortality in smolts of marginal quality.

3. Most of the fish that died at MFS had typical symptoms of BKD.

4. Plasma cortisol concentrations of fish upon arrival at MFS were lower in fish transported in dilute sea water than in those transported in fresh water.

5. Plasma cortisol concentrations were signficantly higher in fall chinook salmon than in spring chinook salmon.

6. Plasma cortisol concentrations reached a peak after transport and had declined to pre-transport levels 96 hours after transport.

7. Survival in sea water at MFS was not correlated with BKD incidence in the hatchery (as measured by DFAT). Incidence of DFAT-positive fish equaled or exceeded 10% in fish of all groups tested in 1983.

6. EFFECTS OF EXPOSURE OF SMOLTS TO STRESS ON RETURN RATE OF ADULTS

The hypothesis that exposure to stressful conditions during collection and transport adversely affects the viability of chinook salmon smolts seems cogent, in light of the known deleterious effects of the stress response. The stress response in fishes is characterized by osmoregulatory dysfunction, increased demand on energy reserves, and impairment of the immune response, However, correlation between exposure to stress and decreased smolt-to-adult survival can be directly tested only by comparison of returns of adults from marked groups of stressed and unstressed smolts.

To test the hypothesized correlation between stress and decreased survival, we proposed to release a group of 80,000 marked chinook salmon smolts from Eagle Creek NFH that had been exposed to simulated collection and transport conditions, along with a marked control (unstressed) group of equal size. The Eagle Creek NFH was chosen because fish released there migrate down the Clackamus and Willamette rivers and enter the Columbia River downstream from Bonneville Dam, and thus are not subjected to either dam passage or collection and transportation.

Marking and release of Eagle Creek chinook salmon was originally planned for spring 1983 (fish of the 1981 brood year). However, disease outbreaks in the groups selected for marking made it inadvisable to use them. Fish of the 1982 brood year were then made available for the test and two groups (80,000 each) were tagged with coded wires in May 1983. Because marked groups of age 0 salmon at Eagle Creek grew rapidly and were relatively large by late summer, the fish were released in October. Fall releases of large,

smolt-like chinook salmon from other Oregon hatcheries in the Willamette River drainage have produced acceptable adult returns.

Methods and Materials

Our objective was to duplicate in the Eagle Creek "stressed" group the profile of changes in plasma cortisol typically observed in fish collected and transported from Lower Granite Dam. Our 1982 baseline studies at Dworshak NFH suggested that we could do this by varying the loading density of fish in the raceways at Eagle Creek over periods of time corresponding to the duration of each successive step in the collection and transportation process at Lower Granite Dam. Loading densities in the raceways were adjusted by crowding fish into the downstream end of raceways with movable screens.

We carried out a preliminary test at Eagle Creek on September 9-11, 1983. The crowding protocol followed was 10 minutes at 2.3 pounds/gallon (simulation of bypass passage from dam to **raceways);** 16 hours at 0.5 pound/gallon (simulation of overnight holding in raceways at Lower Granite Dam); 10 minutes at 2.3 pounds/gallon or actual loading into **a** small (300 gallon) fish tank by hand net (alternative **ways** of simulating truck loading at the dam); 2 hours at 0.5 pound/gallon (abbreviated simulation of truck transport): and release back into raceways. Blood samples for cortisol analyses were taken at intervals during the test from the stressed fish and from undisturbed fish in adjacent raceways. Sampling and cortisol analysis procedures were as described in preceding sections of this report.

A similar protocol was followed during the actual test on October 13-15, 1983. The high-density crowding

alternative was used to simulate truck loading (15 minutes at 2.1 pounds/gallon and simulated truck transport (at 0.5 pound/gallon) lasted for 8 hours.

Results

Nine raceways containing about 73,500 marked fish that were stressed and an additional 73,500 marked fish that were unstressed were involved in the October 13-15 test. These fish (estimated mean fork length 158 mm: W. Zaugg, NMFS, personal communication) were subsequently released on October 17. Because of a misunderstanding with hatchery personnel, we believed that each raceway of fish had received a unique mark, and so matched adjacent raceways as tests and controls (e.g., raceway 9 as test fish, raceway 10 as control fish, etc.). We later learned that groups had been split after marking, so that fish in adjacent raceways had identical microwire tag codes. This invalidated our test: fish released as stressed and unstressed smolts will not be distinguishable when they return to the hatchery as adults. (Fish of the 1982 brood year were later made available to repeat the test; they were marked with oxytetracycline and released in spring 1984.)

Plasma cortisol data from the preliminary test in September indicated that baseline levels were about 8 ng/ml, or about 8%-13% of the baseline level measured in various groups of chinook salmon smolts at Dworshak NFH in spring 1982 (Congleton et al. 1983). Peak levels in fish stressed by crowding were 35-47 ng/ml (Fig. 6.1), in comparison with levels of 120-200 ng/ml typically seen in chinook salmon smolts in the collection and transportation system at Lower Granite Dam. However, peak levels in the Eagle Creek fish represented roughly a 400%-600% rise above baseline levels, compared with a 50%-200% rise above baseline levels typically seen in fish at Lower Granite Dam.

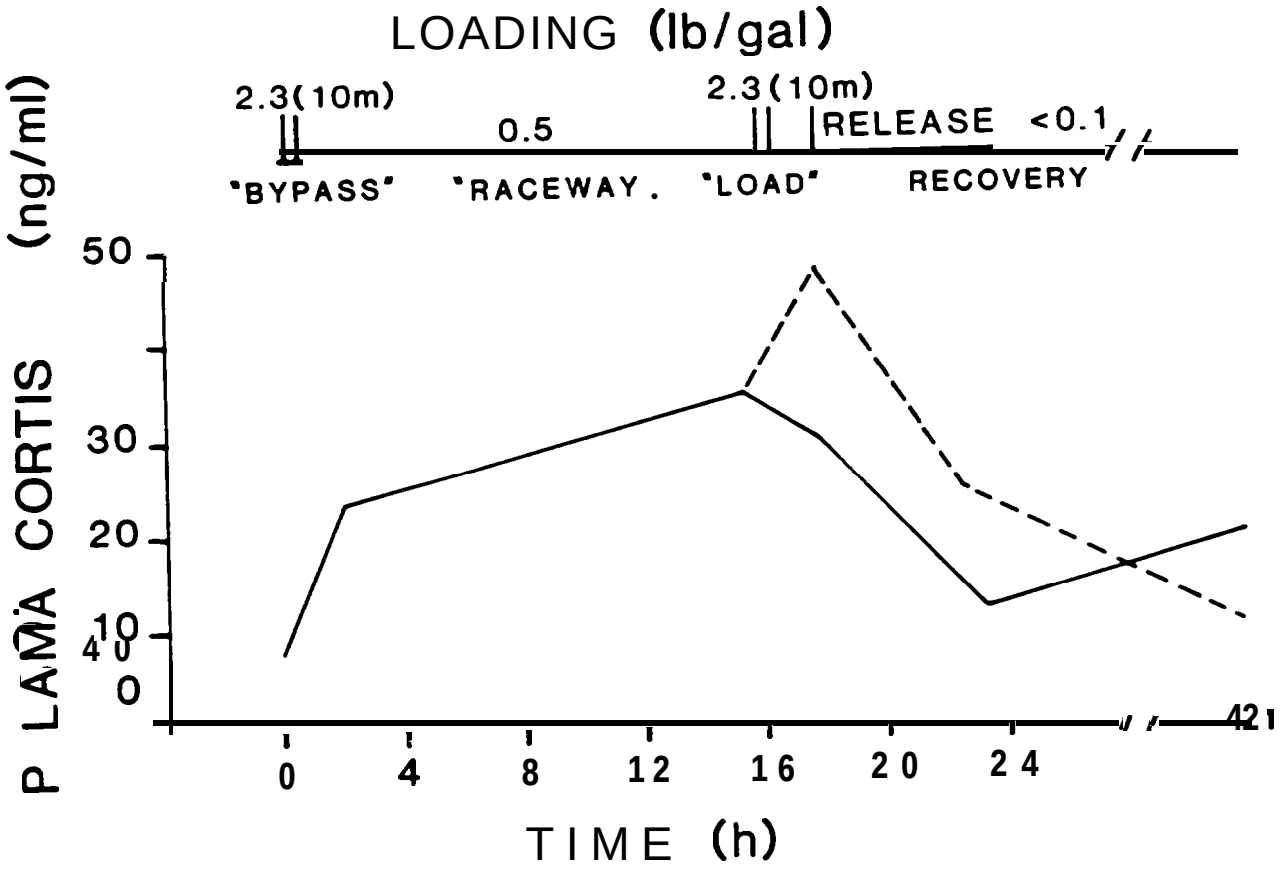


Figure 6.1. Mean plasma cortisol levels in spring chinook subjected to simulated collection and transport by crowding in raceways at Eagle Creek National Fish Hatchery, September 9-14, 1983. The solid line represents fish for which truck loading and transport were simulated by 10 minutes of crowding at 2.3 lb/gallon followed by 2 hours at 0.5 lb/gallon (upper scale). The dottedline represents fish loaded by net into a 200-gallon tank and held 2 hours at 0.5 lb/gallon.

Discussion

A release of stressed and control groups of spring chinook salmon from Eagle Creek NFH was made in spring 1984. Addition of oxytetracycline to the food of these fish is expected to produce distinctive ring patterns, visible under ultraviolet light, on the otoliths of returning adults.

Despite their large size and smolt-like appearance, relatively low baseline cortisol levels indicated that spring chinook salmon released from Eagle Creek NFH in mid October 1983 differed physiologically from chinook salmon smolts that migrate seaward in the spring. Specker (1981) found that plasma cortisol was 8- to lo-fold higher in salmonid smolts than in Parr, and suggested that the spring rise in cortisol may be a useful index of smoltification. We do not know if all chinook salmon that appear and behave like smolts in the fall have low baseline cortisol levels compared with those chinook salmon that became of smolts in spring.

Summary

1. The objective of releasing microwire tagged groups of stressed and unstressed fish was not attained in fall 1983.

2. Groups of stressed and unstressed fish were marked with oxytetracycline and released from Eagle Creek NFH in spring 1984.

3. Cortisol levels in smolt-like chinook salmon sampled at Eagle Creek NFH in September 1983 (8 ng/ml) were similar to levels measured in chinook salmon parr in Idaho hatcheries. Smolt-like fish in Idaho hatcheries

have plasma cortisol levels of 20-60 ng/ml in April and May. This difference could be seasonal or genetic.

7. MODERATION OF STRESS RESPONSE BY TRANSFER OF SMOLTS TO DILUTE SEAWATER

Baseline studies of the response of spring chinook salmon to crowding stress indicated that concentrations of plasma cortisol and glucose declined more rapidly in smolts recovering from crowding stress in 20°/oo sea water than in smolts recovering in fresh water (section 2 of this report). In tests by Long et al. (19771, exposure to salt water in concentrations ranging from 5O/oo to 2o"/oo markedly improved short-term survival of spring chinook salmon transported by truck from Little Goose Dam to Bonneville Dam and subjected to stressful conditions (periodic air exposure) for an additional 24 hours.

In 1983 we undertook tests to determine the effect of different sea water concentrations on plasma cortisol and Na+ levels in spring chinook salmon smolts after transportation and handling. Objectives were to (1) determine whether the stress response would be moderated by exposure to dilute sea water and (2) if so, determine optimal salinities for reduction of the stress response.

Methods and Materials

About 400 spring chinook salmon were transported in a 300-gallon fish transport tank from Dworshak NFH to the University of Idaho on April 13, 1983, and again on April 19. After arrival at the University (1.5-hour transit time) the fish were kept in the transport tank an additional 2.5 hours (total lVtransport'V time, 4 hours). They were then transferred, 5 at a time, into plastic containers holding 20 gallons of fresh water or dilute artificial sea water ("Marine Environment"). Salinities were 0, 5, 10, 15, and 20°/oo, and each salinity was duplicated either four or five times to allow sampling at 2, 6, 12, 24, and 48 hours (2-

hour samples were not taken from the containers with 5 and 15°/oo). After 5 fish had been transferred to each container, 5 more were added to each in succession, until all contained 15 fish. To insure that fish had been adequately stressed, they were suspended out of water for 30 seconds in a dip net as they were being transferred from the transport tank to the plastic container. Procedures for blood sampling and plasma cortisol and Na+ analyses were as described in preceding sections of this report.

Chinook salmon used in the test had been preselected for size at the hatchery and averaged 145 mm in total length (range 130-160 mm). Salmon were held at the hatchery in raceways at 4-5 C, transported in water of 7-9 C, and held in 7-8 C aerated water during the test.

Results

In the first test (April 13-15; Fig. 7.1, Table 7.1), the mean plasma cortisol concentrations after 2 hours of recovery were highest in fish in 0 and 20°/oo salinity (114 and 120 ng/ml cortisol). In fish held at 10°/oo salinity, cortisol concentrations were relatively low (46 ng/ml). After 6 hours of recovery, cortisol concentrations were highest in fish in 20 and 15°/oo sea water, followed by fish in fresh water and those in 5 and 10°/oo sea water. Cortisol concentrations declined during the first 12-24 hours of recovery; no further decrease was seen at 48 hours.

Cortisol concentrations were higher in the second test (April 19-21; Fig. 7.2, Table 7.1) than in the first, but the same groups of fish as in the first test (0 and 10°/oo) had the highest cortisol concentrations after 2 hours of recovery. As in the first test, fish in 15 and 20°/oo sea water had the most elevated cortisol concentrations after 6 hours of recovery, followed by fish in fresh water and

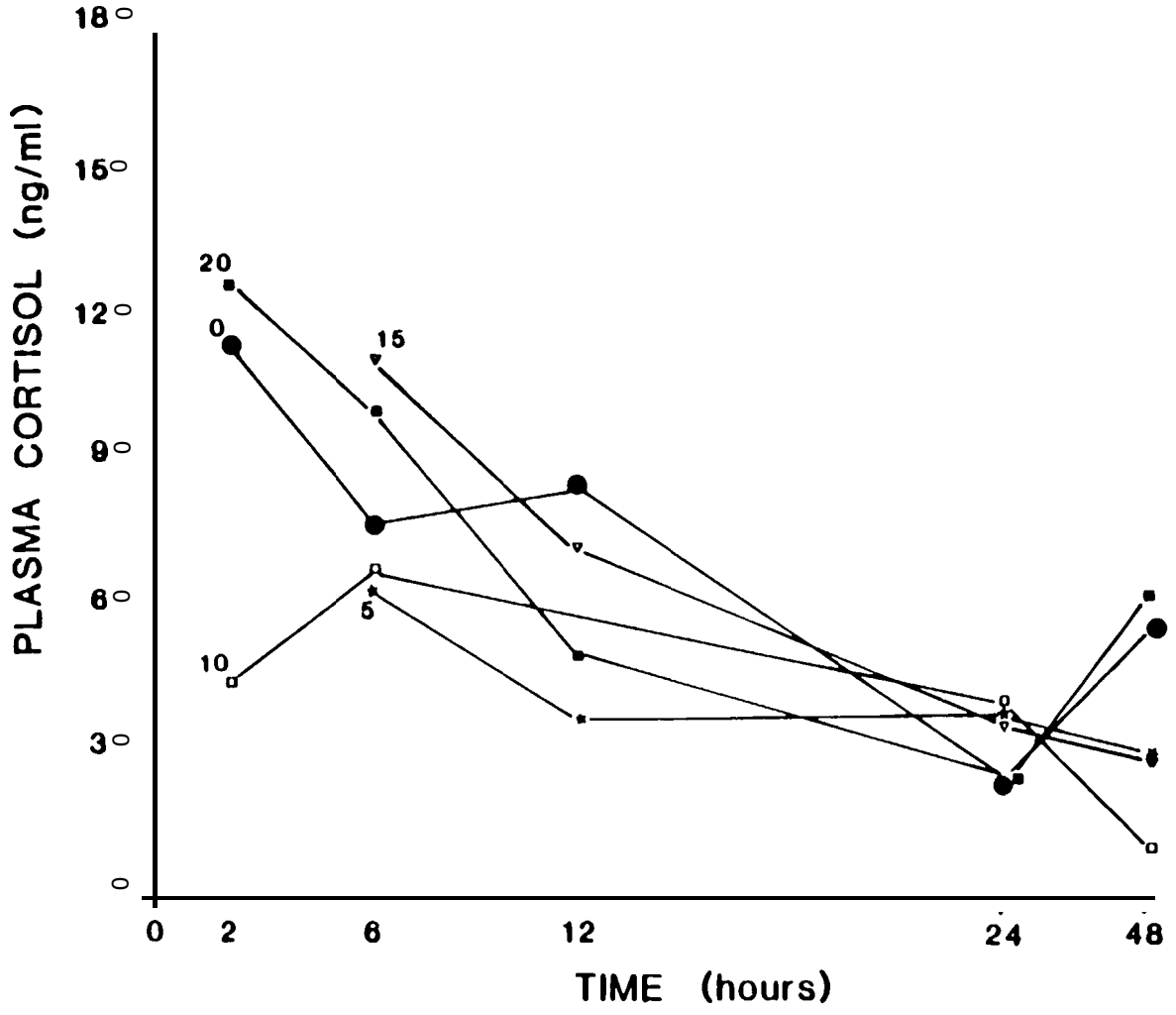


Figure 7.1. Plasma cortisol levels in spring chinook salmon recovering in fresh water and dilute sea water (5, 10, 15, and 20°/oo) from truck transportation and handling (April 13-15, 1983). The fish were unloaded from the truck at 0 hours.

Table 7.1. Mean plasma cortisol concentrations (ng/ml) in spring chinook salmon recovering in fresh water and dilute sea water (5, 10, 15, and 20‰) from truck transportation and handling (April 13-15, 1983). The fish were unloaded from the truck at 0 hours. Ninety-five percent confidence limits for means are in parentheses.

Recovery Time (h)	Salinity (‰) and Trial Number									
	0		5		10		15		20	
	1	2	1	2	1	2	1	2	1	2
2	141 98-184	114 85-142			115 81-148	46 26-65			183 9-228	128 96-160
6	67 49-86	79 53-105	54 38-70	65 6-124	51 29-72	68 99	83 5-109	112 88-1	130 92-168	105 62-150
12	40 26-5	85 40-130	34 6-6	38 25-3	46 25-6		47 26-68	74 54-9	16 0-22	51 36-65
24	34 20-48	26 1-42	20 8-3	42 25-58	35 8-9	40 2-60	39 9-50	37 (9-56	54 6-72	26 0-42
48		57 4-80	26 9-3	1 14-48	26 8-39	1 0-24	43 29-56	30 4-45	53 (42-65	63 9-87

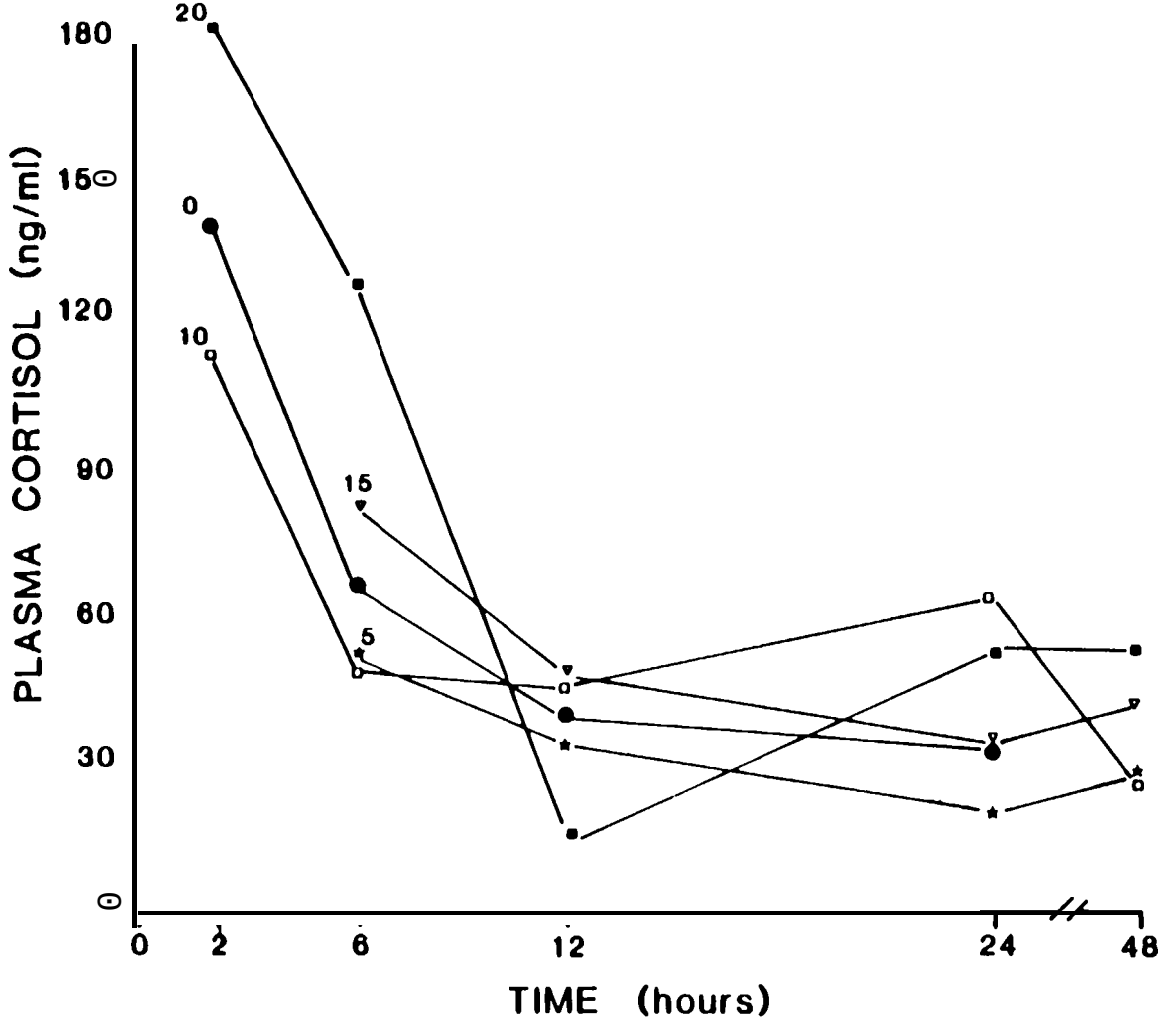


Figure 7.2 Plasma cortisol levels in spring chinook salmon recovering in fresh water and dilute sea water (5, 10, 15, and 20°/oo) from truck transportation and handling (April 19-21, 1983). The fish were unloaded from the truck at 0 hours.

finally by those in 5 and 10°/oo sea water. Cortisol concentrations declined for 12 hours; no further decreases were evident at 24 and 48 hours. The drop in mean cortisol concentration in fish in 20°/oo sea water between 6 and 12 hours was noteworthy: at 6 hours these fish had the highest concentrations (130 ng/ml), and at 12 hours the lowest (16 ng/ml).

Plasma Na^+ concentrations were measured for fish held in 0, 10, and 20°/oo sea water in the April 19-21 test (Fig. 7.3). After 2 hours of recovery, fish in both 0 and 20°/oo sea water had relatively low Na^+ concentrations (138 and 140 meq/liter; at 6 and 12 hours, concentrations in fish in fresh water increased only slightly, whereas concentrations in fish in 20°/oo sea water increased to 151 meq/liter. Plasma Na^+ concentrations in fish in 10°/oo sea water were intermediate between those of fish in 0 and 20°/oo sea water. Plasma Na^- concentrations in all groups ranged from 147 to 152 meq/liter after 24 hours of recovery.

Discussion

Cortisol concentrations determined 2 and 6 hours after handling were highest in chinook salmon held in water with 15 and 20°/oo salinity, somewhat lower in fish held in fresh water, and lowest in fish held in 5 and 10°/oo sea water. Abrupt transfer to 15 and 20°/oo sea water stressed the fish, adding to the cortisol rise resulting from transportation and handling. Conversely, cortisol concentrations were reduced in fish held in salinities of 5 and 10°/oo. Sea water at 10°/oo is approximately isotonic to the blood of fish and the need for osmoregulatory compensation is least at that salinity. Redding and Schreck (1983) reported that coho salmon in isotonic sea water reduced plasma cortisol concentrations more rapidly than did fish in undiluted sea water or fresh water, and suggested

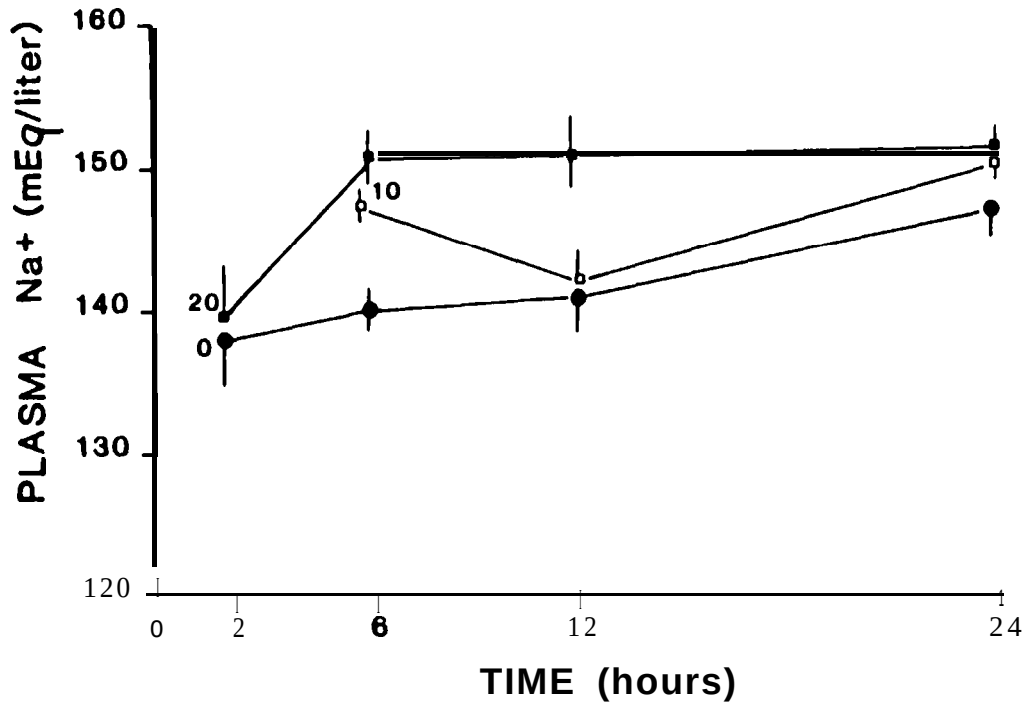


Figure 7.3. Plasma Na^+ concentrations ($\bar{X}$ ± S.E.; n = 12-15) in spriny chinook salmon recovering in fresh water and dilute sea water (5, 10, 15, and 20°/oo) from truck transportation and handling (April 19-21, 1983). The fish were unloaded from the truck at 0 hours.

that cortisol initially increases during stress to facilitate mobilization of energy reserves. If osmotic imbalance is prevented by exposure of fish to isotonic media, energetic needs for osmoregulation are met more easily than would otherwise be true, and cortisol concentrations can decline rapidly.

Relatively low plasma Na^+ levels (138-140 meq/liter) were measured in fish held in 0 and 20°/oo salinity after 2 hours recovery--presumably a consequence of handling diuresis and ion loss during and following transportation. Plasma Na^+ in the 20°/oo group increased to 151 meq/liter after 6 hours of recovery, but rose more slowly in fish held in fresh water. At 20°/oo salinity a positive Na^+ gradient from water to blood would have favored passive Na^+ uptake and a rapid increase in plasma Na^+, soon necessitating initiation of active Na^+ excretion to prevent hypernatremia. In contrast, recovery from a condition of ion depletion in fresh water depends upon energy-requiring branchial ion uptake against a steep blood-to-water Na^+ gradient: consequently, the process is relatively slow.

Disturbance of osmotic and ionic regulation resulting from stress of handling and confinement is believed to have been primarily responsible for the 15%-20% mortality rate commonly seen in groups of chinook salmon held at Bonneville Dam for several days after they had been transported from Snake River dams in the late 1970s (Long et al. 1977). Long et al. (1977) demonstrated that these short-term mortalities could be virtually eliminated by hauling fish in dilute sea water. Release into brackish water following transportation in fresh water should also have a beneficial effect on survival, although this option (hauling in fresh water followed by recovery in dilute sea water) was not included in the study by Long et al. (1977). Delayed (48 hours) mortality rates for chinook salmon smolts hauled to

Bonneville Dam from the Snake River have not been determined in recent years. Improvements in collection and transportation procedures may have reduced short-term mortality rates from those in earlier years. Therefore, short-term mortalities due to osmotic and ionic imbalance during and after transportation should not exceed 10%-20% with current transportation practices and do not appear to be an adequate explanation for the poor return rates of Idaho chinook salmon.

Transportation-related stress may cause delayed mortalities after fish enter the estuary or ocean (e.g., by supression of immune response, resulting in death from disease), but such a delayed effect has not been demonstrated. Cortisol concentrations in transported fish are elevated for 2-4 days, including a recovery interval of 1-2 days after release. The recovery period could be reduced by approximately 1 day for trucked fish if they were transported in salt water and for barged fish if they were released into brackish water in the lower estuary. The benefits of these procedures could be determined by trial releases of marked fish.

Summary

1. Peak plasma cortisol concentrations in spring chinook after truck transportation (4 hours) and handling (unloading by hand net) were reduced when the fish recovered in 5 or 10°/oo sea water rather than in fresh water or in 15 or 20°/oo sea water. The rate of recovery of plasma Na^+ levels was directly related to salinity and was most rapid at 20°/oo.

2. The use of 5 to 10°/oo sea water or salt water in truck transportation would reduce mortality due to osmoregulatory or ionoregulatory disturbances.

Estimates of this mortality have not been made in recent years, but were typically 15%-20% in the early years of the transportation program, and would now presumably be the same (perhaps less).

3. Although the use of salt during barge transportation is not a practical option, release of fish into brackish water in the lower Columbia estuary, rather than into fresh water in the upper estuary, could speed recovery of stress indices to baseline levels. Benefits of accelerated recovery from stress should be determined through trial releases of marked fish.

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